The effect of extrusion with molasses and addition of chitosan or tannins on the rumen undegradable protein fraction of plant protein sources

by

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Master of Agricultural Sciences at **Stellenbosch University**
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Declaration

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Date: December 2020

Dedication

To my beloved late father,

Vivian Jordaan.

(15/09/1962–26/11/2019)

Thank you for empowering me.

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Notes

The language and style used in this thesis are in accordance with the requirements of the South African Journal of Animal Science (March 2020). This thesis represents a compilation of manuscripts, where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

Results of Chapter 3 and Chapter 4 of this thesis were presented as posters at national congresses:

“Brand, T.S., Jordaan, L. & Brundyn, L., 2017. The effect of extrusion on the rumen undegradable protein fraction of lupins. 50th South African Society for Animal Science Congress, 18-21 September.”

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Chapter 4 of this thesis is under review at Animal Feed Science & Technology. “Brand, T.S. & Jordaan, L., 2020. The effect of extrusion with molasses on the rumen undegradable protein fraction of canola oilcake meal and crushed sweet lupins.”

Summary

Title	:	The effect of extrusion with molasses and addition of chitosan or tannins of the rumen undegradable protein fraction of plant protein sources
Candidate	:	Leanne Jordaan
Supervisor	:	Prof. T.S. Brand
Institution	:	Department of Animal Sciences, Stellenbosch University
Degree	:	MSc Agric (Animal Science)

Protein is one of the most expensive nutrients in livestock diets. Therefore, it is essential to pursue the efficiency of protein utilisation in ruminant diets. The inclusion of plant protein sources such as lupins and oilcakes in ruminant diets is limited due to high rumen degradable protein (RDP) content as it does not supply enough rumen undegradable protein (RUP) and amino acids for high producing ruminants. One way of improving nitrogen and thus protein efficiency may be to reduce the dietary protein degradation in the rumen, thereby increasing the proportion of RUP. Therefore, by protecting the protein from degradation in the rumen, it would increase the supply of amino acids to the small intestine. This could also reduce nitrogen wastage through excretion in urine, which renders more protein, especially essential amino acids, available for absorption to increase animal production parameters including growth, milk and wool production. The process of extrusion and the addition of a polymer (such as chitosan) or a polyphenol (such as tannins) have shown potential to reduce the rumen degradability of plant protein sources to increase the nutritional value thereof for ruminants. The aim of the current study was, therefore, to increase the RUP fraction of plant protein sources (lupins, canola oilcake meal and soybean oilcake meal) through extrusion (hot and cold) with molasses and the addition of a polymer (chitosan) and polyphenols (hydrolysable tannins).

The effect of extrusion and addition of chitosan and tannins on the dry matter (DM) and crude protein (CP) rumen degradability were determined with the *in situ* technique, using Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula. The sheep had *ad libitum* access to clean drinking water and a basal diet of wheat straw and lucerne hay (50:50) during the experimental period. Samples were incubated in the rumen of the sheep in polyester bags at different intervals over several periods during the four different trials.

For the first study, lupin samples of *L. albus* and *L. angustifolius* were extruded at maximum temperature reaching 116 °C. Extrusion lowered the soluble fraction while increasing the potential degradable fraction without affecting the rate of degradation of the potential degradable fraction of CP. Extrusion significantly lowered the effective degradability of CP of both lupins by 28% at an outflow rate of 0.08% per hour. No differences were observed between lupin types. Extrusion was found to modify ruminal degradation parameters of *L. albus* and *L. angustifolius*, while also decreasing the effective rumen degradation, especially at faster outflow rates.

For the second study, the effect of extrusion with 6% molasses at 116 °C was determined with locally produced canola oilcake meal (CM) and crushed sweet lupins (CL). Extrusion significantly lowered the CP soluble fraction of CM by 62.2%. The soluble fraction of CM did not differ from CL (46.0%) and CL did not differ significantly from crushed sweet lupins extruded (CLE, 38.2%). Extrusion increased the CP potential degradable fraction by 43.5%. At each outflow rate, namely 0.02, 0.04, 0.05, 0.06 and 0.08/h, the CP effective degradability was lower for CM than for CL. The average effective degradability for CM and CL was 68.2% and 78.0%, respectively. Extrusion significantly lowered the CP effective degradability for both protein sources at every outflow rate tested. The biggest effect was seen at 0.08/h where effective degradation was lowered by 25.6%. Extrusion with molasses was found to modify ruminal degradation parameters of both canola oilcake meal and crushed sweet lupins, while also decreasing the effective rumen degradation, especially at faster outflow rates. Thereby, the combined rumen undegradable protein fraction of canola oilcake meal and crushed sweet lupins was increased by 85.4% through extrusion.

The third study evaluated the effect of cold extrusion with 6% molasses and the addition of 1% chitosan on the protein degradability of soybean oilcake meal. This research showed no differences with cold extrusion or the addition of chitosan and molasses on the rumen undegradable protein fraction of soybean oilcake meal. The benefits of extrusion could not be reached with soybean oilcake meal and cold extrusion as applied in this study.

The fourth study evaluated the effect of cold extrusion with 6% molasses and the addition of 1% hydrolysable tannins on the protein degradability of soybean oilcake meal. This research showed no differences with cold extrusion with molasses and the addition of 1% hydrolysable tannins on the rumen undegradable protein fraction of soybean oilcake meal. The benefits of extrusion could not be reached with soybean oilcake meal and cold extrusion as applied in this study.

The RUP fraction of lupins and canola oilcake meal was increased by extrusion with molasses in this study, and therefore it could be included more efficiently in ruminant diets. This study showed that the benefits of extrusion could be reached at a relatively lower temperature of 116 °C to reduce the chance of heat damage and possible production cost. The temperatures during cold extrusion might have been too low to elicit the desired effects. Furthermore, the addition of 1% chitosan or tannins might have been too low to elicit the desired protein binding effect. Even though no significant differences were seen in this study by cold extrusion or addition of chitosan and tannins, the literature shows that chitosan and tannins have great potential as a feed additive by binding protein. However, more research is needed to fully understand the mode of action of chitosan and tannins in the rumen and the bioavailability of bound protein in the small intestine. The possibility for further improvement still exists by adjusting the processing conditions of extrusion and method of including different additives. Achieved results in the first two studies should also be tested in a biological study to determine the availability of amino acids in the RUP fractions.

Opsomming

Titel	:	The effect of extrusion with molasses and addition of chitosan or tannins of the rumen undegradable protein fraction of plant protein sources
Kandidaat	:	Leanne Jordaan
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Proteïene is een van die duurste voedingstowwe in vee-diëte. Daarom is dit noodsaaklik om doeltreffendheid van proteïenbenutting in herkouerdiëte na te streef. Die insluiting van plantproteïenbronne soos lupiene en canola oliekoeke in herkouerdiëte is beperk weens die hoë rumen-afbreekbare proteïen (RDP) -inhoud, aangesien dit nie genoeg rumen-onafbreekbare proteïene (RUP) en aminosure lewer vir hoë produserende herkouers nie. Een manier om stikstof en dus proteïeneffektiwiteit te verbeter, kan wees om die proteïenafbreking in die rumen te verminder en sodoende die verhouding RUP te verhoog. As gevolg van die beskerming van die proteïen teen afbraak in die rumen, sal dit die toevoer van aminosure na die dunderm verhoog. Dit kan ook die vermorsing van stikstof deur uitskeiding in uriene verminder, wat meer proteïene, veral noodsaaklike aminosure, beskikbaar stel vir opname en dus tot verhoging in die produksieparameters van diere kan lei, soos groei, melk en wolproduksie. Die proses van ekstrusie en die toevoeging van 'n polimeer (soos chitosan) of 'n polifenol (soos tanniene) het potensiaal getoon om die rumen afbreekbaarheid van plantproteïenbronne te kan verminder om die voedingswaarde daarvan vir herkouers te verhoog. Die doel van die huidige studie was dus om die RUP-fraksie van plantproteïenbronne (lupiene, canola-oliekoekmeel en soja-oliekoekmeel) te verhoog deur ekstrusie (warm en koud) met molasse en die toevoeging van 'n polimeer (chitosan) en polifenole (hidroliseerbare tanniene).

Die effek van ekstrusie en toevoeging van chitosan en tanniene op die droë materiaal (DM) en ruproteïen (CP) rumen afbreekbaarheid is bepaal met behulp van die *in situ* tegniek, met behulp van Dohne Merino hammels van ± 80 kg, toegerus met rumen kanule. Die skape het *ad libitum* toegang gehad tot skoon drinkwater en 'n basiese dieet van koringstrooi en lusernhooi (50:50) gedurende die eksperimentele periode. Monsters is gedurende die vier verskillende proewe met verskillende tussenposes gedurende verskillende periodes in die rumen van die skape in poliëstersakkies geïnkubeer.

In die eerste studie is lupien monsters van *L. albus* en *L. angustifolius* by 'n maksimum temperatuur van 116 °C geëkstrudeer. Ekstrusie het die CP oplosbare fraksie verlaag terwyl die potensiële afbreekbare fraksie vergroot was sonder om die afbrekingstempo van die potensiële afbreekbare fraksie te beïnvloed. Ekstrusie het die effektiewe afbreekbaarheid van CP van albei

lupiene met 28% verlaag teen 'n deurvloeitempo van 0,08% per uur. Geen verskille is waargeneem tussen tipes lupiene nie. Daar is gevind dat ekstrusie die rumen afbreekparameters van *L. albus* en *L. angustifolius* verander, terwyl dit ook die effektiewe rumen afbreekbaarheid verminder, veral teen vinniger deurvloeitempos.

In die tweede studie is die effek van ekstrusie met 6% molasse by 116 °C bepaal met plaaslik vervaardigde canola-oliekoekmeel (CM) en fyngedrukte soetlupiene (CL). Ekstrusie het die CP-oplosbare fraksie van CM aansienlik verlaag met 62,2%. Die oplosbare fraksie van CM verskil nie van CL nie (46,0%) en CL verskil nie beduidend van geëkstrudeer fyngedrukte soetlupiene nie (CLE, 38,2%). Ekstrusie het die CP potensiële afbreekbare fraksie met 43,5% verhoog. By elke deurvloeitempo, naamlik 0,02, 0,04, 0,05, 0,06 en 0,08 / h, was die CP effektiewe afbreekbaarheid laer vir CM as vir CL. Die gemiddelde effektiewe afbreekbaarheid vir CM en CL was onderskeidelik 68,2% en 78,0%. Ekstrusie het die CP effektiewe afbreekbaarheid vir beide proteïenbronne aansienlik verlaag teen elke getoetsde deurvloeitempo. Die grootste effek is gesien by 0,08 / h, waar effektiewe afbreekbaarheid met 25,6% verlaag is. Daar is gevind dat ekstrusie met molasse die rumen afbreekparameters van beide canola oliekoekmeel en fyngedrukte soetlupiene verander, terwyl dit ook effektiewe afbreekbaarheid in die rumen verminder, veral teen vinniger deurvloeisnelhede. Daardeur is die rumen se onafbreekbare proteïenfraksie van canola oliekoekmeel en fyngedrukte lupiene gesamentlik met 85,4% verhoog deur ekstrusie.

Die derde studie het die effek van koue ekstrusie met 6% molasse en die toevoeging van 1% chitosan op die proteïenafbreekbaarheid van sojaboon oliekoekmeel geëvalueer. Hierdie navorsing het geen verskille getoon met koue extrusie of die toevoeging van chitosan en molasse op die rumen onafbreekbare proteïenfraksie van sojaboon oliekoekmeel nie. Die voordele van ekstrusie kon nie bereik word met sojaboon oliekoekmeel en koue ekstrusie soos toegepas in hierdie studie nie.

Die vierde studie het die effek van koue ekstrusie met 6% molasse en die toevoeging van 1% hidroliseerbare tanniene op die proteïenafbreekbaarheid van sojaboon oliekoekmeel geëvalueer. Hierdie navorsing het geen verskille getoon met koue ekstrusie met molasse en die toevoeging van 1% hidroliseerbare tanniene op die rumen onafbreekbare proteïenfraksie van sojaboon oliekoekmeel nie. Die voordele van ekstrusie kon nie bereik word met sojaboon oliekoekmeel en koue extrusie soos toegepas in hierdie studie nie.

Die RUP fraksie van lupiene en canola oliekoekmeel is in hierdie studie deur ekstrusie met molasse verhoog, en daarom kan dit doeltreffender ingesluit word in herkouerdiëte. Hierdie studie het getoon dat die voordele van ekstrusie bereik kan word by 'n relatief laer temperatuur van 116 °C om die kans op hittebeskadiging en moontlike produksiekoste te verminder. Die temperature tydens koue extrusie kon te laag gewees het om die gewenste effekte te bewerkstellig. Verder sou die toevoeging van 1% chitosan of tanniene te laag gewees het om die gewenste proteïenbindingseffek te bewerkstellig. Alhoewel geen noemenswaardige verskille in hierdie studie deur koue extrusie of toevoeging van chitosan en tanniene waargeneem is nie, toon die literatuur dat chitosan en tanniene

tog potensiaal het om proteïen te bind. Daar is egter meer navorsing nodig om die werking van chitosan en tanniene in die rumen en die biobeskikbaarheid van gebonde proteïene in die dunderm ten volle te begryp. Die moontlikheid vir verdere verbetering bestaan steeds deur die verwerkingstoestande van extrusie en die metode om verskillende bymiddels in te sluit, aan te pas. Bereikte resultate in die eerste twee studies moet ook in 'n biologiese studie getoets word om die beskikbaarheid van aminosure in die RUP fraksies te bepaal.

List of abbreviations

A	:	The rapidly soluble fraction; represent 0 hour disappearance
ADF	:	Acid detergent fibre
ANOCOVA	:	Analysis of covariance
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
B	:	The fraction that will degrade over time; potential degradable fraction
BL	:	Broad-leaf *Lupinus albus*
C	:	Calcium
C	:	The rate of degradation of the *B* fraction
CF	:	Crude fat
CL	:	Crushed sweet lupins
CLE	:	Crushed sweet lupins extruded
CM	:	Canola oilcake meal
CME	:	Canola oilcake meal extruded
CP	:	Crude protein
Deg	:	Degradability at time *T*
Deg_{eff}	:	Effective degradability
DM	:	Dry matter
e	:	Natural logarithm
IARC	:	The International Agency for Research on Cancer
k		Rate of passage or Fractional outflow rate of RUP from the rumen
LS	:	Least squared (mean)
NL	:	Narrow-leaf *Lupinus angustifolius*
NRC	:	National Research Council
P	:	Phosphorus
RDP	:	Rumen degradable protein

RUP	:	Rumen undegradable protein
SAS	:	Statistical Analysis System
SE	:	Standard error
SOM	:	Soybean oilcake meal with molasses
SOMC	:	Soybean oilcake meal with chitosan
SOMCE	:	Soybean oilcake meal with molasses and chitosan and extruded
SOME	:	Soybean oilcake meal with molasses extruded
SOMT	:	Soybean oilcake meal with molasses and tannins
SOMTE	:	Soybean oilcake meal with molasses and tannins and extruded
T	:	Time

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Chapter 1
General Introduction

Over the years, animal scientists have focused their research on increasing animal production to keep up with the ever-growing population, which leads to increased demand for animal products. This increasing demand, together with increased consumer awareness of health, ethical and environmental issues, has led researchers to focus more on animal production efficiency and strategies for optimisation. One such strategy is the strategic use of nutrition for enhancing production (Martin & Kadokwa, 2006). Improving productivity of livestock and meeting future demands for animal products and food security can be achieved if high producing animals are fed according to their specific nutrient requirements to reach their genetic potential (González *et al*., 2018).

The increased demand for animal products and the competition for plant protein products used for human consumption may lead to the availability of protein sources for animal production becoming limited or cost prohibitive (Manceron *et al*., 2018). In addition, geopolitical crises and weakening economies in certain regions may limit or prevent the trade of protein sources. These limitations could be especially detrimental in parts of the world where soybeans (the most popular plant protein source in animal feeds) do not grow or where soybeans and soybean meals have to be imported. Therefore, using available local plant protein alternatives efficiently and having processing sites nearby can contribute to increased feed security (Albin, 2015). Nutritionists are continuously researching products that could optimise animal production traits by improving the diets of the animals and enhancing the value of feed ingredients, while reducing environmental impact and keeping costs as low as possible (Haraki *et al*., 2018).

Protein is one of the most expensive nutrients in livestock diets. Therefore, it is essential to pursue the efficiency of protein utilisation. One of the main problems with high producing ruminants is the excess of rumen degradable protein (RDP) and a deficiency of rumen undegradable protein (RUP) content (Davidović *et al*., 2019). One way of improving nitrogen and thus protein efficiency may be to reduce dietary protein degradation in the rumen, thereby increasing the proportion of RUP, also called bypass protein. Therefore, by protecting the protein from degradation in the rumen, it would increase the supply of amino acids to the small intestine and could reduce nitrogen wastage through excretion in urine, which renders more protein, especially essential amino acids, available for absorption to increase animal production parameters including growth, milk and wool production (Mohamaden *et al*., 2020).

Protein sources high in RUP is expensive, scarce and for the most part unpalatable (for example fishmeal or bloodmeal). Plant protein sources, especially alternatives to soybean meal, including lupins or by-products such as oilcakes, are usually high in RDP. For instance, canola oilcake meal and lupins can partially replace soybean meal in ruminant diets, but inclusion is

restricted due to high rumen degradable protein (RDP) contents of these ingredients (77 and 81%, respectively; NRC, 2001). Even soybean oilcake meal poses the ability to be further processed to reduce the RDP content thereof. The most common method used for decreasing RDP content of plant protein sources is heat treatment. Drawbacks of this method include the fact that processing sites could be expensive, as high heat is needed over periods of time. It is also challenging to keep conditions constant, which might lead to overheating or under heating of the raw material. Alternative methods to increase the RUP fraction of feed receiving attention more recently are the addition of natural additives. The addition of antibiotics and formaldehyde to increase the RUP fraction of feed has raised health concerns for the consumer. The available information in the literature on natural additives is growing, and some of these additives are used in the feed industry, for example, tannins. The potential exists to explore further options that have been touched on in literature, such as chitosan, but it has not been implemented in the feed industry. Different sources of tannins and chitosan have shown potential for decreasing RDP and thus leading to increased RUP and improved animal performance. The optimal dose has not been approved as it varies greatly, especially between animal species and plant protein sources, giving contradicting results. Feed dictionaries lack data on the effect of processing on the nutritional value of feed. Chitosan has not been studied much, and hydrolysable tannins are not widely used in ruminant nutrition yet. Although studies have evaluated the effect of chitosan and tannins on rumen fermentation, few studies have evaluated the effect of dietary chitosan and tannin inclusion on the rumen degradability parameters through *in situ* trials. The potential exists to specifically look at the effects of the combination of extrusion with molasses and the addition of chitosan or tannins on rumen degradability of plant protein sources.

It is important to note that for animal performance to be improved, the RUP (especially amino acids) should be bioavailable in the small intestine for absorption. The effect on animal performance will also depend on the amount as well as the amino acid profile. Processing and binding could render protein unavailable and it will be excreted.

In order to optimise diet formulation, accurate data is required on ruminal protein degradation of plant protein sources. This data could be added to feed dictionaries, to be used in diet formulations for ruminants using processed plant protein sources. Underutilised plant protein sources could be used more efficiently after processing or treatment. An increase in nitrogen efficiency in ruminants will lead to less wastage and no need to overfeed protein, which generally has cost implications.

The aim of the current study was, therefore, to evaluate different techniques to increase the RUP fraction of plant protein sources (lupins, canola oilcake meal and soybean oilcake meal), for example extrusion (hot and cold) with molasses and the addition of a polymer (chitosan) or polyphenols (hydrolysable tannins).

The objectives were to:

- Determine the effect of extrusion on the *in situ* rumen degradability of locally produced lupins.
- Determine the effect of extrusion with molasses on the *in situ* rumen degradability of locally produced canola oilcake meal and crushed sweet lupins.
- Determine the effect of cold extrusion with molasses and the addition of chitosan on the *in situ* rumen degradability of soybean oilcake meal.
- Determine the effect of cold extrusion with molasses and the addition of hydrolysable, sweet chestnut tannins on the *in situ* rumen degradability of soybean oilcake meal.

Each objective is addressed separately in the respective research chapters.

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Chapter 2
Literature Review

2.1 Introduction

Ruminant production contributes to sustainable food safety due to the ability of ruminant animals (such as sheep and cattle) to convert feed and by-products, that is often of low quality and has little to no value for human food, into high quality protein products that are available for human consumption (Broderick, 2018). Strategies for increasing animal production could include, but is not limited to, improved feed digestion, improving the feed conversion ratio and increasing the dietary nutrient density of feed, but this could be difficult with increasing feed costs (Haraki *et al*., 2018). Former strategies included over formulation for protein in ruminant diets to ensure enough amino acids reach the absorption sites in the small intestine. This overfeeding of protein results in wastage. The excess is excreted, which increases production costs and is related to environmental issues of nitrogen pollution. There is a need to optimise available animal feeds in terms of protein quality and quantity, in ways which will increase animal production efficiency and profitability as well as decrease nitrogen waste in the environment (Garg, 1998; González *et al*., 2018; Haraki *et al*., 2018). This chapter will further explore how ruminants metabolise dietary proteins and ways of improving the available plant protein sources to optimise its use in ruminant nutrition.

2.2 Protein metabolism in ruminants

The efficiency of protein utilisation in ruminants is a function of protein digestion in the rumen and post rumen tract, absorption of the digested proteins through the rumen wall or intestinal villies, and metabolism in the organs and different tissues of the animal. Ruminants have a symbiotic relationship with rumen micro-organisms (microbes). The host animal supplies the microbes with an optimum environment with constant food supply and remove fermentation end products (carbon dioxide and methane) and unfermented feed from the rumen. The main constituents of ruminant feed consist of carbohydrates (fibre, sugar, starch) and proteins (Chalupa, 1975). A diagrammatic representation of the ruminant digestive system showing the utilisation of dietary carbohydrates, protein and urea (Brand, 1996) is presented in Figure 2.1. The microbes ferment digestible carbohydrates to volatile fatty acids (VFA), which is an important source of energy to the host animal. Dietary protein is divided into the rumen degradable protein (RDP) fraction and the rumen undegradable protein (RUP) fraction. The RDP fraction is soluble and broken down by rumen microbes, turning it into ammonia, which is the primary nitrogen source for microbial protein synthesis. Microbial protein supplies the majority of amino acids to the absorption sites in the small intestine to be utilised by the host animal (Annonier *et al*., 2001; Chiang *et al*., 2009). The RUP fraction is a smaller part of dietary protein that escapes degradation in the rumen and thus passes

intact to the abomasum. The amino acids derived from RUP continue towards the small intestine (specifically the duodenum), which is the main absorption site (Solanas *et al*., 2008), where it is available for further metabolism in different animal tissues, such as meat, wool or milk (Walli, 2005; Makkar & Beever, 2013). The RUP fraction (also called bypass protein) and especially the profile of the essential amino acids reaching the small intestine, is thus a significant factor in determining the protein value of feeds for ruminants (Goiri *et al*., 2009c).

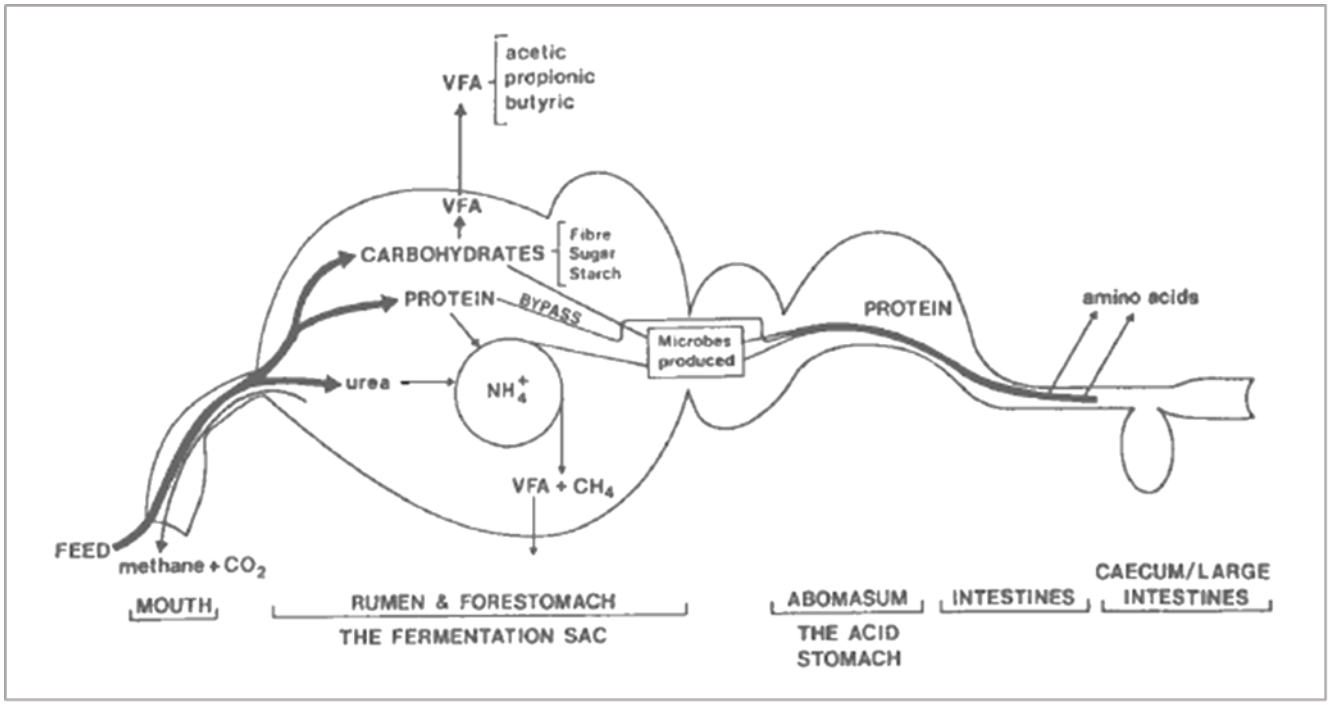

Figure 2.1 Diagrammatic representation of a ruminant digestive system showing the utilisation of dietary carbohydrates, protein, and urea (Brand, 1996).

Overfeeding of protein sources high in RDP, which exceeds the requirement of the rumen microbes, leads to inefficient use of good quality RDP. As rumen microbes break down RDP, it leads to large scale ammonia production which is absorbed into the bloodstream of the host animal. It, however, requires energy to be converted to urea in the liver; much of which is excreted through the urine (McDonald, 1948). Only a small portion of urea is recycled in the rumen to contribute nitrogen that will be used to produce microbial protein. This phenomenon results in an increased cost of production and environmental concerns relating to nitrogen pollution (Savari *et al*., 2018). Non-protein nitrogen could be supplied in diets more economically and just as effective by using urea for microbial protein synthesis in the rumen and therefore lowering the cost of urea synthesis (Chalupa, 1975). It also leads to increased quality of amino acids reaching the small intestine from more expensive RUP sources, which has been shown to increase animal production through growth and milk production (Shelke *et al*., 2012). Metabolisable protein is thus the protein reaching the small intestine from microbial protein and RUP. Completely undegradable protein reaches the small

intestine, but is not available for absorption. Thus, undegraded protein is excreted and does not contribute to the animal's protein requirement.

2.3 Protein requirements

Ruminant protein requirement is not constant, and it depends on the changing physiological and productive state of the animal (Kempton *et al*., 1977). Microbial protein reaching the small intestine makes up 40-80% of total protein reaching this area, depending on animal species and production level. Microbial protein is usually sufficient to meet the maintenance requirements of ruminants if energy is sufficiently supplied. However, in high producing animals such as young growing animals or lactating females, the microbial protein from RDP is insufficient, and the amount it contributes towards protein requirement is low, therefore, a larger amount of RUP is needed to meet metabolisable protein requirements (NRC, 2001; Erickson *et al*., 2016). To reach the full genetic potential of the animal the diet needs to provide sufficient RDP to supply in the needs of the rumen microbial population and sufficient RUP to escape rumen fermentation to supply additional amino acids to the absorption sites in the small intestine. Ruminants still need adequate NPN in their diets to ensure efficient microbial protein synthesis (Garg, 1998). The ratio of RDP:RUP depend on various factors including animal species and production level. The diet of a high producing cow could include 60% to 70% of RDP and 30% to 40% RUP (Kaldmäe *et al*., 2010). The RUP fraction is mainly the limiting factor in production of dairy cattle, while RDP is usually adequate or excessive. Therefore, a need exists to further investigate different ways to increase the RUP fraction of plant protein sources.

Most studies show that diets containing higher amounts of ruminally undegradable proteins or ruminally protected amino acids resulted in increased milk production, while other studies show little or no response. According to Schingoethe (1996), the lack of response to RUP is often due to one of the following reasons: (i) the rumen may have been bypassed at the expense of ruminal microbial protein synthesis; (ii) the RUP may have been poorly digested postruminally; and (iii) the RUP may have been deficient in the essential amino acids that are limiting production.

2.4 Protection of protein from ruminal degradation

Strategies for protecting RDP have been developed over five decades and was sparked by the discovery of the Maillard reaction. Some of these strategies include reducing rumen degradability of protein sources and its solubility in rumen fluid, the duration the protein is retained in the rumen or using essential amino acid analogs or encapsulation thereof (Chalupa, 1975). Solubility can be decreased by processing or coating of protein sources or changing the activity of the rumen microbes. Decreasing retention time in the rumen will be dependent on factors affecting the rate of passage of digesta, including: dry matter intake, specific gravity, the particle size of feed, concentrate

to roughage ratio and rate of rumen digestion dependent on animal species and production level (Chalupa, 1975).

A good source of RUP should be able to remain stable in the rumen, where the pH is in the range of 5.5 to 7.0, for an extended period and then permits quick release within a short period in the abomasum and small intestine, where the pH drops to 3.5. It is, however, important to note that the pH and rate of passage depend on different factors such as diet (Church, 1979; Annonier *et al*., 2001).

Different methods of increasing the RUP of available plant protein sources include physical treatments such as heat treatment (feed manufacturing, extrusion, jetsploding, dry roasting, autoclaving) and chemical treatments (binding or coating of protein molecules to chemicals such as formaldehyde, tannins, polymers, essential oils, yeast) or a combination thereof. Formaldehyde and heat treatment are the most widely used and accepted methods for increasing the RUP fraction of plant protein sources and has the potential to be economical. Formaldehyde binds proteins by the formation of methylene bridges, which makes them resistant to microbial attack. However, concerns were raised with using formaldehyde as it is believed to be carcinogenic and could render a human health risk (IARC, 2004). Currently, there is renewed interest in natural additives that can be used (Patra & Saxena, 2009).

Extrusion as heat treatment

Most feed processing techniques usually leads to increased heat and can be used as a method of heat treatment during manufacturing or drying of feed. Animals utilising heat treated feeds have shown better growth and milk production mainly due to decreased RDP, but also the destruction of anti-nutrients in the feed (Kaufman & Lupping, 1982; Van Dijk *et al*., 1983). Extrusion has been used in human food and animal feed industries for many years. The extrusion process applies heat and pressure in the presence of moisture. This process is most commonly known for oil extraction, but also more recently to decrease rumen degradability of protein sources (White *et al*., 2007; Zagorakis *et al*., 2015). The raw material is fed through a barrel with increasing pressure as the barrel tapers towards the outlet. This method of protecting proteins is considered safe and economical. The physical characteristics of the feed are altered as extrusion promotes starch gelatinisation and partial Maillard reaction, which improves the durability of feed rations (Chang & Wang, 1999; Svihus *et al*., 2005; Solanas *et al*., 2008). This process, also referred to as extrusion cooking, causes denaturing of proteins, which decreases protein solubility and thus also decrease the ruminal degradability of protein in feeds (Barchiesi-Ferrari & Anrique, 2011). The RUP fraction is thus increased, providing greater quantities of amino acids available for absorption (Solanas *et al*., 2008).

Processing conditions can alter the quality of the protein in the feed. Van Soest (1987) suggested that the optimum heat input depends on the characteristics of the plant protein source, namely moisture content, carbohydrate content and composition, protein content and presence of

sulphate. Moderate heat damage can be beneficial to decrease RDP without compromising digestibility and availability of RUP entering the small intestine, with typical temperatures used of 130–180 ºC and more (Walli, 2005; Solanas *et al*., 2008). Temperatures above 180 ºC may lead to over-heating, resulting in irreversible adverse effects of heat damaged proteins, decreasing the digestibility of RUP and rendering it completely biologically unavailable for absorption to the animal (Kung & Rode, 1996). Besides temperature, the duration the feed is exposed to this heat and pressure and the amount of moisture added, all contribute to the characteristics of the end product. The optimal conditions for extrusion of plant protein sources are difficult to be established as it is challenging to keep processing conditions constant. In addition, very little information about the processing conditions is supplied in the studies in literature, which makes interpretation of results difficult.

In previous studies it was shown that heat treatments, including autoclaving and extrusion at temperatures from 100 ºC to 150 ºC, showed a decrease in rumen degradability of soybean, oilcakes, lupins and grains, thus increasing the RUP fraction (Ljøkjel *et al*., 2000; Griffiths, 2004; Solanas *et al*., 2008). Some studies, however, found no effect of heat treatment or extrusion even at high temperatures (above 100 ºC) of soybean meal and soybean oilcake meal (Keery *et al*., 1993; Deacon *et al*., 1988).

The Maillard reaction is likely to occur due to the heat, moisture, and pressure. Thus, the addition of molasses might be beneficial to get better results. In 1975, Chalupa suggested that the Maillard reaction between sugar aldehyde groups and free amino groups can be controlled to decrease protein degradability in the rumen, without adversely affecting intestinal protein digestibility. The treatment of soybean meal and canola oilcake meal with xylose was successful in decreasing the RDP fraction thereof (Cleale *et al*., 1987; Harstad & Prestløkken, 2000; Tuncer & Sacakli, 2003). Paula *et al*. (2017) added 2-3% molasses to canola meal before extrusion to increase the browning reaction.

Processing costs can be expensive due to the high heat used. The potential exists to lower production cost if the same result can be achieved at lower temperatures. However, too low temperatures might lead to under-heating which will have no effect on RUP. Literature on cold extrusion used in ruminant diets is scarce.

Addition of chitosan

Chitosan is derived from deacetylated chitin found in the exoskeletons of insects, crustaceans and molluscs and the cell walls of fungi and certain algae (Li *et al*., 2018). It is a non-toxic, biodegradable biopolymer and is the second most abundant polysaccharide in nature after cellulose (Li *et al*., 2018). Chitosan has received attention for its diverse potential application in medicine, food and cosmetics and is seen as a new feed additive in ruminant diets, primarily because of its antimicrobial activity (Del Valle *et al*., 2017). Chitosan is available in a range of different molecular weights and degree of acetylation (Terbojevich *et al*., 1993). The different physicochemical

characteristics thereof result in different antimicrobial activities and chemical properties (Mima *et al*.,1983; Rhoades & Roller, 2000). It has been used in the food industry to keep food from spoilage with a longer shelf life.

Chitosan is insoluble at a pH above 6 and dissolves readily below a pH of 6, which means, theoretically, if it binds to protein it has the potential to stay stable in the rumen environment and could reverse its bond with proteins for absorption in the small intestine. When chitosan dissolves in acid, it becomes a gel-like substance, which has the potential to coat feed particles. Fadel El-Seed *et al*. (2003) used chitosan as a nitrogen source for rumen microbes as it contained 6.7% nitrogen, but found that it was not degraded in the rumen and suggested that it could perhaps be used as a RUP source for ruminants. Theoretically, the characteristics of chitosan could make it a good source of RUP, as chitosan is known to be readily soluble at pH below 6, depending on the degree of deacetylation (Rinaudo, 2006), and as the pH increases above 6 it becomes insoluble (Pillai *et al*., 2009).

Ruminant nutrition studies using chitosan have given variable results. However, several studies have been shown to change ruminal fermentation by shifting volatile fatty acid profiles, including higher propionate concentration and lower acetate to propionate ratio, which most likely improve the energy efficiency of ruminal fermentation and reduced methane production in ruminants (Goiri *et al*., 2009a, b, 2010; Haryati *et al*., 2019; Seankamsorn *et al*., 2020). Mingoti *et al*. (2016) found a reduction in faecal nitrogen excretion with chitosan supplementation, which might be related to improvement in protein digestibility. There seems to be a lack of agreement between studies, resulting in insufficient conclusive information. The optimal dose in diets still needs to be clarified, so it is currently not widely used in ruminant nutrition.

Addition of tannins

Tannins are complex, naturally occurring plant polyphenolic compounds that have the potential to protect proteins from ruminal degradation and to decrease the rate of ammonia build-up in the rumen (Henke *et al*., 2017; Aderao *et al*., 2020). Tannins can form reversible bonds with proteins, but these bonds are stable within the rumen pH range (5.5 to 7.0). Tannins are less susceptible to degradation in the rumen by inhibiting the growth and activity of proteolytic bacteria, thereby increasing the quantity of proteins that reach the abomasum and small intestine (Patra & Saxena, 2011; Henke *et al*., 2017; Patra & Aschenbach, 2018; Davidović *et al*., 2019; Sarnataro & Spanghero, 2020). The tannin-protein bond is believed to segregate at low pH, which occurs in the acidic abomasum or the duodenum and so allows a higher absorption of proteins and amino acids in the intestine (Chalupa, 1975; Jones & Mangan, 1977). The suppressing effect of tannins on the rumen microbiome links its value to environmental issues, not only through reducing nitrogen pollution, but also decreasing methane emissions from rumen fermentation (Patra & Saxena, 2011; Patra & Aschenbach, 2018; Sarnataro & Spanghero, 2020). The effect of tannins on the rumen microbiome and the protein binding capacity depends on the structure and source of tannins as well as the plant

protein source (Giner-Chavez *et al*., 1997; Kraus *et al*., 2003; Zeller *et al*., 2015). Tannins could be classified into two main groups, namely condensed tannins and hydrolysable tannins, which are different in structure and molecular weight depending on the origin thereof (Mohamaden *et al*., 2020; Sarnataro & Spanghero, 2020). Condensed tannins are the most intensively studied for its use in decreasing rumen degradable protein fractions and improving nitrogen utilisation, but also for reducing bloat and parasitism in ruminants and reducing methane emissions (Coblentz & Grabber, 2013). The concern with regard to condensed tannins is that the bond with proteins might sometimes be irreversible as it is more stable in the rumen environment and not degraded by natural processes, rendering the protein unavailable for absorption in the small intestine (Archana *et al*., 2010; Mezzomo *et al*., 2015). Hydrolysable tannins have a weaker bond with proteins and it may be degraded in the rumen with metabolites being absorbed into the bloodstream, which could lead to toxicity (Khanbabaee & Van Ree, 2002; Aboagye & Beauchemin, 2019). However, studies are showing no detrimental effect by using hydrolysable tannins in ruminant diets, while some authors recorded no differences in degradability of protein sources when adding hydrolysable or condensed tannins (Driedger & Hatfield, 1972; Getachew *et al*., 2008; Liu *et al*., 2011). It has been shown that high tannin content in diets (>5% DM) reduces voluntary intake and nutrient digestibility through decreased feed palatability and slower digestion (Frutos *et al*., 2004a; Mueller-Harvey, 2006). By contrast, the intake of diets with low to medium tannin content (1-4% DM) has been shown to improve feed conversion and digestion, mainly due to decreased ruminal protein degradation (Mueller-Harvey, 2006; Patra & Saxena, 2011). However, the effect of tannins on protein degradability is inconsistent.

Several authors found positive effects with diets containing condensed tannins in dairy cows, such as increased milk yield and milk protein as well as decreased milk urea nitrogen concentration (Dey & De, 2014; Wang *et al*., 1996; Soltan, 2009; Allam *et al*., 2013; Anantasook *et al*., 2015). Other researchers found that the inclusion of 2-4% condensed tannins (Piñeiro-Vázquez *et al*., 2017) or hydrolysable tannins (Wischer *et al*., 2014) did not affect protein utilisation efficiency in cattle and sheep, respectively. Arisya *et al*. (2019) found that tannins from various sources decreased rumen degradability of protein sources, but it did not affect total protein digestibility. They concluded that 2% chestnut tannin in diets gave the best results. Availability of literature on condensed tannins is extensive, whereas hydrolysable tannins have been less studied in ruminant nutrition. Small quantities of hydrolysable tannins in feed are shown to be neither toxic nor have adverse effects on animal production (Frutos *et al*., 2004a). There have been studies showing improved animal performance parameters and protein degradability using feed with the addition of hydrolysable tannins (Hervás *et al*., 2000, Frutos *et al*., 2004b, Mohamaden *et al*., 2020, Sarnataro & Spanghero, 2020). More research is needed on hydrolysable tannins as it is readily degradable in the rumen and the effects thereof could be nullified. The potential further exists to use combinations of hydrolysable and condensed tannins. The cost of the use of tannins depends on the source used and method of extraction.

2.5 Techniques for evaluating protein quality of feed

Wide variations in both protein sources and animal species differences, make it very difficult to accurately measure the protein degradability in the whole digestive system of the animal. Many techniques have been developed to provide reasonable estimates of the degree of digestibility and degradability of raw materials. These include *in vivo, in situ* and *in vitro* techniques that are developed to quantify ruminal degradation of feeds more accurately and precisely.

In vivo method

In vivo methods are used for total flux of digesta through the digestive tract of live animals by using markers in the feed. The fundamental assumption is that an animal is in a nitrogen equilibrium, nitrogen pools stay steady and that turnover rates remain constant during the study, which is near impossible to attain practically. *In vivo* methods are useful for testing the productive performance and feeding trials, which is helpful in tested products or feed that has been established to improve protein degradability. However, testing new products needs a smaller scope, where the focus can be put on specifically the rumen and the small intestine. The *in vivo* method is labour intensive, the turnover rate is slow and is not practical for testing feeds on large scales as large quantities of feed are needed. This technique can also not be accurately used for single feedstuffs as the animals need to eat a balanced diet meeting their specific requirements.

In situ method

In situ methods use a combination of the ideas from *in vivo* and *in vitro* methods. It is the use of live animals with test samples inside dacron bags that can be retrieved from incubation in the rumen or post ruminal cannula over time. Ørskov & McDonald (1979) developed a model by using this method to obtain an estimated rate of degradation in the rumen according to passage rate. The assumption is that the environment inside the bag will resemble the surrounding rumen environment. The method is not without shortcomings as the bag characteristics, feed particle size, animal species and production all limit the results. However, it is commonly accepted and used as it is seen as the most effective method of doing rumen degradability studies, especially when comparing feeds or treatments. The reproducibility is low, mainly due to variability between animals, and thus the results from using this method might not apply to all situations. The method is undesirable due to its implications on animal welfare due to needing surgically prepared animals and the costs thereof, and so there is a limited sample size and number of samples that can be incubated at the same time. Thus, there is great interest in developing convenient alternative and cheaper *in vitro* methods.

In vitro method

In vitro methods are based on the solubility of protein as an index of digestibility (Stern & Satter, 1982). Feed samples are incubated in rumen fluid in an environment that simulates the rumen with regards to heat and movement, mostly using a water bath, and degradability can be measured at different incubation time points (Tilley & Terry, 1963). The gas production technique is an alternative,

but it has been criticised for using a waste product of fermentation to evaluate feedstuffs and is thus not a direct representation of the extent of degradability. Another *in vitro* method is the Ross Assay (Ross *et al*., 2013) that simulates the digestion in the rumen and then further in the small intestine by addition of enzymes to lower the pH of the rumen fluid. *In vitro* methods are great alternatives to *in vivo* methods and decrease the number of animals needed, although rumen fluid is still needed. It is costly, laboratory equipment is needed, and a good understanding of the method is crucial as the chemicals used are essential to the accuracy of the results. Good correlation is mostly seen between *in vitro* and *in situ* studies, but more studies are needed to credit the method as an alternative for the *in situ* method. Less cannulated animals are needed for *in vitro* studies as it is only necessary to get rumen fluid once per period and a large number of samples can be done in one period compared to the *in situ* method which is limited to samples per animal. There still exists a need for alternative methods that do not require surgically prepared animals and resembles true degradation more closely than current synthetic enzymes.

2.6 Plant protein sources

Feed versus food competition leads to the search for alternatives plant protein sources and to enhance or improve the utilisation of the available sources. With changing climates and an ever-growing world population with increased consumer awareness of the environmental impact and health issues (animals and humans), there is a need to look at alternative protein sources and methods to use current sources as efficiently as possible.

In order to increase the efficiency of protein utilisation from the highly degradable protein sources, these proteins need to be protected from excessive ruminal degradation, allowing the protein to bypass the rumen (rumen undegradable protein, RUP).

Plant protein sources make up the second largest proportion of livestock diets (following energy sources), of which soya is the most common plant protein source used (Tona, 2018). Soya for animal feed is facing market competition with human food demands, especially in developing countries (Mengesha, 2012). This feed-food competition led to the necessity to explore the use of locally available, cheaper alternative protein sources for use in livestock feed formulations (Tona, 2018).

Only a few feeds are good sources of RUP and are naturally high in RUP such as bloodmeal, fishmeal, maize gluten meal, cottonseed oilcake, coconut oilcake and maize grain. These sources are mostly expensive, scarce and make feed unpalatable. Sources of medium protein degradability include linseed oilcake and deoiled rice bran. Soybean meal, mustard oilcake, groundnut oilcake, sunflower seed oilcake, lupins and canola oilcake meal are highly rumen degradable protein sources.

Certain oilcakes are of limited use in ruminant diets due to its high RDP content, for example soybean oilcake (74% RDP), canola oilcake (79% RDP) and lupins (80% RDP) (Erasmus *et al*., 1988).

Soybean oilcake meal

Plant protein sources make up the second largest proportion of livestock diets, of which soybean oilcake meal is the most common plant protein source used because of its high protein content (52.6% DM, INRA-CIRAD-AFZ, 2020) and favourable amino acid composition (Tona, 2018). Soybean is known to be variable in quality, and the potential exists to further process soybean oilcake meal to reduce its rumen degradability for an improved quality protein source to ruminants (Dozier & Hess, 2011).

Lupins

Lupins are legumes cultivated in regions with Mediterranean climates, mostly where monoculture was previously practised (Brand *et al*., 1992). Lupins have been cultivated with great success in the Western Cape area of South Africa for many years, especially in rotation cycles to prevent monoculture. Lupins can be considered to be a local, cheaper, alternative protein source compared to imported soybean meal for use as a source of protein and energy in livestock feeds. Sweet lupins are largely free of anti-nutritional factors and have a low risk of causing acidosis due to low starch levels and high fermentable carbohydrates (Dixon & Hosking, 1992). The relatively high crude protein content of lupins (34%, Brand *et al*., 2004) makes it a valuable resource for ruminant nutrition as they are also cost competitive. The protein in lupins is, however, highly rumen degradable (80%, INRA-CIRAD-AFZ, 2020), compared to soybean oilcake meal (40% CP and 78% RDP, INRA-CIRAD-AFZ, 2020). It is therefore currently not included in large quantities in ruminant diets (Brand *et al*., 1992; Tuncer & Sacakli, 2003; Wright *et al*., 2005; Boguhn *et al*., 2008). This mean that for lupins to be used optimally as the primary source of protein in diets for highly productive ruminants, it must be treated to reduce rumen degradability (Dijkstra *et al*., 2005).

Canola oilcake meal

Canola oilcake meal is a by-product of oil extraction from canola seeds and is commonly incorporated in ruminant rations as a protein supplement due to its desirable amino acid profile (Newkirk *et al*., 2003; Santos, 2011). The protein content of canola meal differs depending on variety, growth conditions and oil extraction method, but it is accepted as having a high crude protein content of 36-40% (Paula *et al*., 2018). Solvent and expeller oil extracted canola meal produced in South Africa contain 31.6% and 42.8% CP respectively on a dry matter basis (Brand *et al*., 2001).

2.7 Hypotheses

It is hypothesised that the RUP fraction of lupins, canola oilcake and soybean oilcake meal will be increased respectively by certain processes of extrusion and the addition of specific dosages of chitosan and tannins, thus potentially increasing the supply of amino acids to the small intestine for improved animal production.

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Chapter 3
The effect of extrusion on the rumen undegradable protein fraction of lupins

3.1 Abstract

Lupins are highly degradable in the rumen and do not provide enough bypass protein to high producing ruminant animals. The effect of extrusion on the dry matter (DM) and crude protein (CP) rumen degradability of *Lupinus albus* and *Lupinus angustifolius* was determined *in situ*. Lupin samples of both types were extruded at maximum temperature reaching 116 °C. Six Dohne Merino wethers, fitted with rumen cannula, were used in this trial. Samples were incubated in the rumen at intervals of 0, 2, 4, 12, 36 and 48 hours. Treatments were randomly assigned to sheep during each period. This procedure was repeated in two sheep per treatment and in three periods, giving a total of six observations for each variable studied. Extrusion lowered the soluble fraction while increasing the potential degradable fraction without affecting the rate of degradation of the potential degradable fraction of crude protein. Extrusion significantly lowered the effective degradability of crude protein of both lupin types by 28% at an outflow rate of 0.08% per hour. No differences were observed with regard to lupin types. Extrusion was found to modify ruminal degradation parameters of *L. albus* and *L. angustifolius*, while also decreasing the effective rumen degradation, especially at faster outflow rates. Thereby, the rumen undegradable protein fraction of lupins is increased by extrusion, and therefore lupins can be used more efficiently in ruminant diets. This study showed that the benefits of extrusion could be reached at a relatively low temperature of 116 °C to reduce the chance of heat damage.

3.2 Introduction

Plant protein sources make up the second largest proportion of livestock diets (following energy sources), of which soybean oilcake meal is the most common plant protein source used (Tona, 2018). Soya has been facing market competition with human food demands, especially in developing countries (Mengesha, 2012). This feed-food competition led to the necessity to explore the use of locally available, cheaper alternative protein sources for use in livestock feed formulations (Tona, 2018).

Optimising the supply and demand of specific nutrients and their requirements for high producing animals is essential to achieve target performance, profitability, product characteristics and environmental outcomes (González *et al*., 2018). As a result, the animal's efficiency would be increased and nitrogen waste would potentially be decreased. Overfeeding protein sources high in rumen degradable protein (RDP) results in wastage as microbes break down RDP into ammonia and excess is excreted as urea in urine. An advantage of feeding rumen protected protein is the

opportunity for utilising non-protein nitrogen for microbial protein synthesis in the rumen and therefore, also lowering the cost of urea synthesis (Shelke *et al*., 2012). Over-formulation of protein has led to overfeeding of protein. This leads to nitrogen losses through excretion and high cost of diets. With changing climates and an ever-growing world population with increased consumer awareness of the environmental impact and health issues (animals and humans), we need to look at alternative protein sources and use current sources as efficiently as possible.

Dietary protein that escapes degradation in the rumen (rumen undegradable protein, RUP, or bypass protein) and reaches the small intestine is a significant factor in determining the protein value of feed for ruminants. The small intestine (specifically the duodenum) is the main site for protein digestion and absorption (Solanas *et al*., 2008). The amount of microbial protein produced from RDP is mostly insufficient for the protein requirements of high producing animals (Erickson *et al*., 2016). Increasing the amount of RUP reaching the small intestine can be done by artificially protecting the dietary protein from degradation in the rumen (Jolazadeh *et al*., 2015; Ouellet & Chiquette, 2016).

Lupins are legumes cultivated in regions with Mediterranean climates, especially where monoculture was previously practiced (Brand *et al*., 1992). Lupins have been cultivated with great success in the Western Cape area of South Africa for many years. Lupins can be considered to be a local, cheaper, alternative protein source compared to imported soybean meal. Lupins are characterised by high grain productivity, it is adapted to poor and barren low pH soils, it has fewer nitrogen fertiliser requirements than other crops and it is suggested to be cheaper than oilseed proteins (Abraham *et al*., 2019). Lastly, it is easy to store and handle due to its durable hull (Abraham *et al*., 2019). Even though lupins have high crude protein values (35%, INRA-CIRAD-AFZ Feed Tables, 2020) it is also highly rumen degradable (80%, INRA-CIRAD-AFZ Feed Tables, 2020), compared to soybean oilcake meal (40% CP and 78% RDP, INRA-CIRAD-AFZ Feed Tables, 2020). It is therefore currently not included in large quantities in ruminant diets (Brand *et al*., 1992; Tuncer & Sacakli, 2003; Wright *et al*., 2005; Boguhn *et al*., 2008). Therefore, in order for lupins to be used optimally as main protein source in diets for highly productive ruminants, it has to be treated to reduce rumen degradability (Dijkstra *et al*., 2005). *Lupinus* is a truly diverse genus with many species. However, only four are commercially cultivated, of which the two most commonly used is broad-leaf *Lupinus albus* and narrow-leaf *Lupinus angustifolius* (Abraham *et al*., 2019).

Extrusion has been used in human food and animal feed industries for many years. Extrusion is a process in feed manufacturing where heat and pressure are applied in the presence of moisture, most commonly for oil extraction but also more recently to decrease rumen degradability of protein sources (White *et al*., 2007; Zagorakis *et al*., 2015). The raw material is fed through a barrel with increasing pressure as the barrel tapers towards the outlet. This method of protecting proteins is considered safe and economical. The physical characteristics of the feed are altered as extrusion promotes starch gelatinisation and partial Maillard reaction, which improves the durability of feed rations (Chang & Wang, 1999; Svihus *et al*., 2005; Solanas *et al.,* 2008). Extrusion also causes

denaturing of proteins, which decreases protein solubility and thus also decrease the ruminal degradability of protein in feeds (Barchiesi-Ferrari & Anrique, 2011). The RUP fraction is thus increased, providing greater quantities of amino acids available for absorption (Solanas *et al.,* 2008). Temperatures above 180 ºC may result in negative irreversible effects of damaged proteins and leaving them completely biologically unavailable to the animal. The extrusion process has shown not to compromise protein degradability in the small intestine when processing at temperatures below 180 ºC (Solanas *et al*., 2008). Besides temperature, the time the feed is exposed to this heat and pressure and the amount of moisture added all contributes to the characteristics of the end product. The optimal conditions for extrusion of lupins have not been established.

The extrusion of lupins poses the possibility of better utilisation of an underutilised plant protein source (van Barneveld, 1999). This may lead to lupins playing a larger role as a main protein source in ruminant feeds. Feed databases lack data on the effect of processing on protein sources, so data obtained by studies may aid in formulating diets.

The aim of this study was to evaluate the effect of extrusion on the dry matter (DM) and crude protein (CP) ruminal degradability of broad-leaf *Lupinus albus* and narrow-leaf *Lupinus angustifolius*.

3.3 Materials and Methods

Animals

Ethical clearance for this research was granted by the Animal Care and Use Research Ethics Committee of the University of Stellenbosch (Ethical clearance number #0378 and #0379). Six Dohne Merino wethers with average live mass of ±80 kg, previously fitted with rumen cannula, were used in this trial. The sheep were housed in enclosed individual pens (1 m x 2 m) at the Welgevallen Experimental Farm of the University of Stellenbosch for the duration of the trial. The sheep had *ad libitum* access to clean water and were supplied a basal diet of wheat straw and lucerne hay (50:50) *ad libitum* during the experimental period. The feed was replenished twice daily (every morning and evening) as necessary. Daily intake was estimated as 3% of the body weight of the sheep. The sheep were already adapted to the feed before the *in situ* trial started.

Treatments

Locally produced seed of broad-leaf *Lupinus albus* (BL) and narrow-leaf *Lupinus angustifolius* (NL) were sourced. Half of the seed was kept raw and the other half extruded with an Insta Pro 2000RC extruder at a commercial feed mill, with temperatures reaching a maximum of 116 °C. The four types of lupin seeds tested in this trial were *L. albus* not extruded (BL), *L. albus* extruded (BLE), *L. angustifolius* not extruded (NL) and *L. angustifolius* extruded (NLE). Samples were milled through a 1 mm screen size using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to be used for further analysis.

In situ evaluation of ruminal degradability

The dry matter (DM) and crude protein (CP) degradability of the broad-leaf and narrow-leaf lupins (control and extruded) were determined by the *in situ* technique described by Ørskov & McDonald (1979). The lupin seeds were dried in a force draught oven for a minimum of 48 hours at 60 °C, after which 5 g samples were weighed off on a digital scale and inserted into a series of previously dried, weighed and marked polyester bags (10 cm x 11 cm) with an average pore size of 53 µm. The bags were tied off by nylon strings of different lengths to prevent the strings from knotting in the rumen and to ensure easy retrieval. Bags were incubated in the rumen for different time intervals, being 2, 4, 12, 36 and 48 hours, with an all-out approach. An incubation series started when the first bag (representing the 48 hour interval) was inserted into the rumen cannula at 09h00 in the morning. As the incubation period shortened, bags were added to the rumen. The incubation was ended when all the bags were removed at the same time after 48 hours. After removal, the bags were submerged in ice water to rapidly stop further degradation. The bags were then washed under running tap water until water squeezed from it was clear. The 0 hour bag was prepared in the same way and was washed under the tap like the rest without being placed in the rumen. All bags were dried after incubation in a force draught oven for a minimum of 48 hours at 60 °C. Treatments were randomly assigned to sheep during each period. This procedure was repeated in two sheep per treatment and in three periods as a completely randomised design, giving a total of six observations for each variable studied.

Chemical Analysis

After drying the bags for 48 hours at 60 °C, the nylon strings were removed, and the dried bags were weighed to determine the DM residue. The nitrogen content (%) of the residue was then determined using the Dumas combustion method (Method 990.03; AOAC, 2002) using a LECO TruMac N Nitrogen Determinator, version 1.3X (LECO Corporation, Michigan, USA). The CP content of the dry matter was determined by multiplying the percentage nitrogen by a factor of 6.25.

Statistical analysis

Dry matter and CP disappearances were expressed as percentages of the amount remaining after rumen incubation. The percentage material degraded was fitted to the following one-compartment model, as proposed by Ørskov & McDonald (1979), by non-linear regression procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2014) to determine DM and CP degradability parameters:

$$\mathrm{Deg} = A + B\,(1 - \mathrm{e}^{-CT})$$

Where Deg = degradation at time, *T* (%)

A = rapidly soluble fraction; represent 0 hour disappearance (%)

B = the fraction that will degrade over time; potential degradable fraction; asymptote (%)

C = the rate of degradation of the *B* fraction (%/h)

Ruminal retention time affects the extent of degradation and therefore a fractional outflow rate of undegraded protein from the rumen (*k*) was taken into account when the percentage effective degradation (Deg_{eff}) was calculated as: $Deg_{eff} = A+BC/(C+k)$ at chosen *k* values: 0.02 (low intake level), 0.04, 0.05 (medium intake level), 0.06, 0.08/h (high intake level).

Non-linear parameters *A*, *B* and *C*, as well as the Deg_{eff} values, were submitted to a two-way analysis of variance (ANOVA) using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2014). Significance was declared at $P \leq 0.05$ and tendencies at $P < 0.10$ using Bonferroni tests.

3.4 Results

The *in situ* DM disappearance parameters for the effect of extrusion on *L. albus* and *L. angustifolius* are summarised in Table 3.1. An interaction was observed in the dry matter soluble fraction with BL being the highest (58.7%), which differed significantly from NL (51.5%), NLE (45.2%) and BLE (44.6%). No interaction was observed for the dry matter potential degradable fraction and the rate of degradation of the potential degradable fraction estimates for DM degradability. No significant difference was observed for the dry matter potential degradable fraction between lupin type, while extrusion increased the potential degradable fraction of the two lupin types by 38%, from 42.4% to 58.5% ($P < 0.001$). No differences were found for the dry matter rate of degradation of the potential degradable fraction with regards to lupin type nor for the effect of extrusion processing.

Table 3.1 The effect of extrusion on the LS means (± SE) *in situ* dry matter rumen disappearance non-linear parameters of *L. albus* (broad-leaf) and *L. angustifolius* (narrow-leaf) seeds

		*Dry matter non-linear parameters		
		A	***B***	***C***
Lupin type	*L. albus*	51.7 ± 1.3	49.7 ± 3.1	0.099 ± 0.02
	L. angustifolius	48.3 ± 0.9	51.2 ± 2.3	0.076 ± 0.02
	P-value	0.051	0.691	0.412
Processing	Not extruded	55.1^{1} ± 0.9	42.4^{2} ± 2.4	0.075^{2} ± 0.02
	Extruded	44.9^{2} ± 1.2	58.5^{1} ± 3.0	0.100^{1} ± 0.02
	P-value	<0.001	<0.001	0.382
Lupin type x Processing	*L. albus* not extruded	58.7^{a} ± 1.3	38.8^{b} ± 3.3	0.078^{a} ± 0.02
	L. albus extruded	44.6^{b} ± 2.1	60.5^{a} ± 5.3	0.120^{a} ± 0.04
	L. angustifolius not extruded	51.5^{b} ± 1.3	46.0^{ab} ± 3.3	0.073^{a} ± 0.02
	L. angustifolius extruded	45.2^{b} ± 1.2	56.5^{a} ± 3.0	0.080^{a} ± 0.02
	P-value	0.026	0.168	0.535

**A* = rapidly soluble fraction (%), *B* = the fraction that will degrade over time (%), *C* = the rate of degradation of the *B* fraction (%/h)

[a,b;1,2] LS means in columns with different superscripts differ ($P < 0.05$)

The calculated dry matter effective degradability (Deg_{eff}) for all treatments at various outflow rates can be seen in Table 3.2. No significant differences in dry matter Deg_{eff} between lupin types as well as processing method were observed at any outflow rate for dry matter disappearance. The average Deg_{eff} (irrespective of lupin type or processing method) at each outflow rate (0.02, 0.04, 0.05, 0.06 and 0.08 per hour) was respectively, 86.9%, 80.1%, 77.7%, 75.7% and 72.5%.

Table 3.2 The effect of extrusion on the LS means (±SE) *in situ* dry matter effective degradation from the rumen of *L. albus* (broad-leaf) and *L. angustifolius* (narrow-leaf) seeds

		***Dry matter effective degradation at fractional outflow rate (%)**				
		0.02/h	**0.04/h**	**0.05/h**	**0.06/h**	**0.08/h**
Lupin type	*L. albus*	86.8 ± 1.4	80.4 ± 2.1	78.2 ± 2.3	76.4 ± 2.5	73.6 ± 2.6
	L. angustifolius	87.1 ± 1.0	79.8 ± 1.5	77.2 ± 1.7	75.0 ± 1.8	71.5 ± 1.9
	P-value	0.892	0.813	0.714	0.637	0.523
Processing	Not extruded	88.4 ± 1.0	82.6 ± 1.6	80.4 ± 1.8	78.5 ± 1.9	75.5 ± 2.0
	Extruded	85.5 ± 1.3	77.7 ± 2.1	75.1 ± 2.3	72.9 ± 2.4	69.6 ± 2.5
	P-value	0.104	0.085	0.087	0.089	0.088

*n=12

For *in situ* crude protein disappearance, significant interactions were observed for the soluble fraction, as well as the potential degradable fraction, and main effects could not be interpreted. The crude protein parameters are summarised in Table 3.3. It is evident that extrusion lowered the crude protein soluble fraction (P <0.05) in both lupin types with respectively 60.3% for the broad-leaf lupins and 36.9% for the narrow-leaf lupins. The potential degradable fractions were increased by respectively 323.5% for the broad-leaf lupins and 103.5% for the narrow-leaf lupins. No interaction or differences were observed between lupin type or method of processing for the rate of degradation of the potential degradable crude protein fraction.

Table 3.3 The effect of extrusion on the LS means (± SE) *in situ* crude protein rumen disappearance parameters of *L. albus* (broad-leaf) and *L. angustifolius* (narrow-leaf) seeds

		*Crude protein non-linear parameters		
		A	***B***	***C***
Lupin type	*L. albus*	56.5 ± 1.1	45.6 ± 2.7	0.153 ± 0.1
	L. angustifolius	55.8 ± 0.8	43.7 ± 1.9	0.246 ± 0.1
	P-value	0.616	0.576	0.352
Processing	Not extruded	74.7^{1} ± 0.9	23.1^{2} ± 2.0	0.260 ± 0.1
	Extruded	37.6^{2} ± 1.1	66.1^{1} ± 2.6	0.139 ± 0.1
	P-value	<0.001	<0.001	0.226
Lupin type x Processing	*L. albus* not extruded	80.9^{a} ± 1.2	17.4^{b} ± 2.9	0.177 ± 0.1
	L. albus extruded	32.1^{d} ± 1.9	73.7^{a} ± 4.5	0.129 ± 0.1
	L. angustifolius not extruded	68.4^{b} ± 1.2	28.9^{b} ± 2.9	0.344 ± 0.1
	L. angustifolius extruded	43.1^{c} ± 1.1	58.8^{a} ± 2.6	0.148 ± 0.1
	P-value	<0.001	0.001	0.453

**A* = rapidly soluble fraction (%), *B* = the fraction that will degrade over time (%), *C* = the rate of degradation of the *B* fraction (%/h)
$^{a\text{-}d;1,2}$ Means in columns with different superscripts differ (*P* <0.05)

The crude protein effective degradability (Deg_{eff}) calculated at the various rates of passage from the rumen is presented in Table 3.4. No marked differences were observed between lupin types at any outflow rate. Extrusion however significantly lowered the effective degradability of crude protein at every outflow rate tested (between 9.5% and 28.0%). The effect of extrusion at the various outflow rates is also visually presented in Figure 3.1. Extrusion had the most pronounced effect at the highest outflow rate (0.08/h) where the reduction in crude protein effective degradability was 28.0%.

Table 3.4 The effect of extrusion on the LS means (±SE) *in situ* crude protein effective degradation from the rumen of *L. albus* (broad-leaf) and *L. angustifolius* (narrow-leaf) seeds

		*Crude protein effective degradation at fractional outflow rate (%)				
		0.02/h	**0.04/h**	**0.05/h**	**0.06/h**	**0.08/h**
Lupin type	*L. albus*	90.7 ± 1.6	82.7 ± 2.2	80.4 ± 2.4	78.6 ± 2.6	76.0 ± 2.9
	L. angustifolius	91.6 ± 1.3	86.9 ± 1.8	85.1 ± 2.0	83.6 ± 2.2	81.1 ± 2.4
	P-value	0.677	0.157	0.159	0.170	0.194
Processing	Not Extruded	95.7^{1} ± 1.4	94.0^{1} ± 1.9	93.2^{1} ± 2.1	92.6^{1} ± 2.3	91.4^{1} ± 2.5
	Extruded	86.6^{2} ± 1.6	75.6^{2} ± 2.1	72.2^{2} ± 2.4	69.7^{2} ± 2.5	65.8^{2} ± 2.8
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001

^{1,2} Means in columns with different superscripts differ (*P* <0.05)

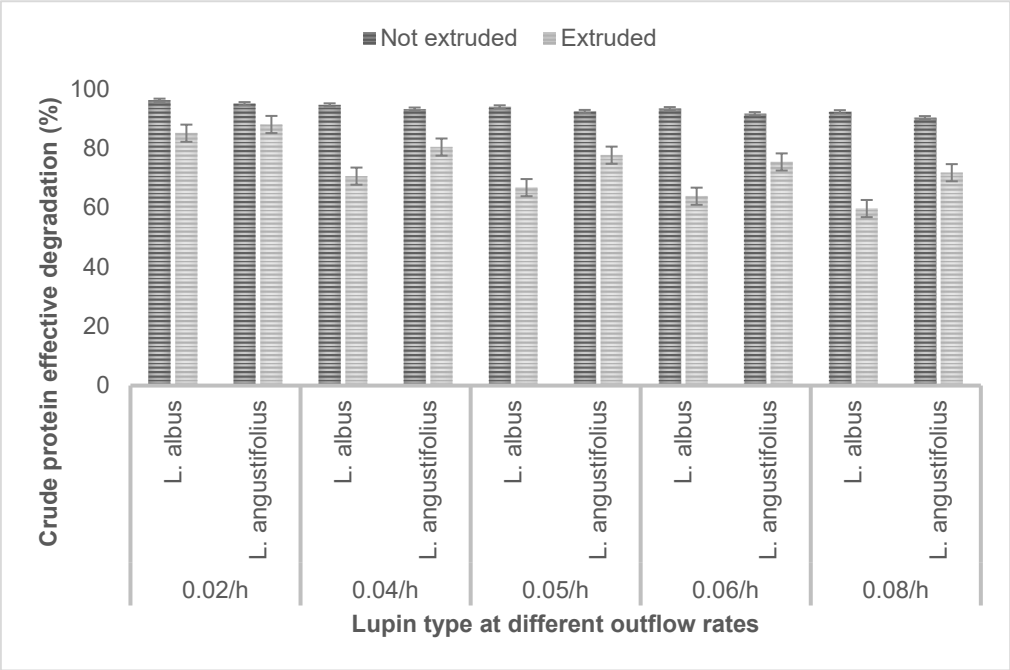

Figure 3.1 The effect of extrusion on the LS mean percentage (%) *in situ* crude protein effective degradation from the rumen of *L. albus* (broad-leaf) and *L. angustifolius* (narrow-leaf) seeds

3.5 Discussion

In this study, extrusion appeared to lower the dry matter soluble fraction and increase the potential degradable fraction with no differences in the rate of degradation of the potential degradable protein fraction. This finding is in agreement with results by Barchiesi-Ferrari & Anrique (2011) who found that extrusion of dehulled *L. albus* at 130 ºC reduced the dry matter soluble fraction by 4.4% (from 36.2% to 34.6%), increased the potential degradable fraction by 0.8% (from 63.9% to 64.4%) and no differences were found in the rate of degradation of the potential degradable fraction. Griffiths (2004) extruded lupins at 115-120 ºC and found the dry matter soluble fraction of lupins was increased with extrusion, which is unexpected. Aufrère *et al*. (2001) found that extrusion increased the dry matter potential degradable fraction of lupins with about 100% compared to the not extruded control. Whilst, Griffiths (2004) did not find any significant difference in the potential degradable fraction. No difference in dry matter effective degradability of the potential degradable fraction was found at 0.08/h outflow rate by Griffiths (2004), which is in line with findings of this study. This means that extrusion did not have an effect on the effective degradability of dry matter at any outflow rate tested despite the effect seen on the degradability parameters.

Extrusion had a marked effect on the crude protein soluble fraction, lowering it by nearly 50% (from 74.7% to 37.6%). This decreased solubility correlates with results reported by other authors. Barchiesi-Ferrari & Anrique (2011) and Barchiesi *et al*. (2018), who extruded dehulled *L. albus* at 120 ºC and 130 ºC respectively at 20% moisture, found a decrease of 12% (42.7% to 37.4%) and 29% (34.2% to 24.4%) respectively in the crude protein soluble fraction. Solanas *et al*. (2008) extruded *L. albus* at 140 ºC with 10% moisture and showed no difference in the crude protein soluble

fraction (average 35%). It seems that non-structural carbohydrates are prone to react with protein in the presence of heat and moisture, diminishing protein solubility at the ruminal level (Solanas *et al*., 2008). Increases in the potential degradable fraction correspond to a reduced soluble fraction of feed (Barchiesi *et al*., 2018).

Extrusion increased the potential degradable crude protein fraction in this study by 186.1% (from 23.1% to 66.1%). Barchiesi-Ferrari & Anrique (2011) found an increase in the crude protein potential degradable fraction of lupins due to extrusion (from 59.3% to 62.2%), but these increases were not biologically significant. Solanas *et al*. (2008) and Barchiesi *et al*. (2018) did not find significant differences, possibly because they used dehulled lupins. Lampart-Szczapa *et al*. (2006) showed lower increases when using dehulled lupins as it has lower moisture absorbance capacity, which probably negatively affect the Maillard reaction.

No differences in the rate of degradability of the potential degradable crude protein fraction was found in this study (average 0.2%). Barchiesi-Ferrari & Anrique (2011) similarly did not find significant differences for the rate of degradability of the potential degradable crude protein fraction (0.09%). Solanas *et al*. (2008) found that extrusion lowered the rate of degradability of the potential degradable crude protein fraction in lupins from 0.248% to 0.124%.

The effect of extrusion on the crude protein effective degradability (Deg_{eff}) of lupins was clearly visible in this study. The greatest decrease of 28% in crude protein degradation was seen at the outflow rate of 0.08/h (91.4% non-extruded to 65.8% extruded). This outflow rate corresponds to 8% per hour and roughly represents an incubation time in the rumen of 12.5 hours, which is generally used as an average value for high-producing cows. In this study extrusion significantly decreased the crude protein effective degradability at outflow rates of 0.02/h (9.5%), 0.04/h (19.6%), 0.05/h (22.5%), 0.06/h (24.7%) and 0.08/h (28.0%). Barchiesi-Ferrari & Anrique (2011) found that extrusion of lupins lowered the crude protein Deg_{eff} at outflow rates as follow: 0.02/h (2.1%), 0.05/h (2.4%) and 0.08/h (2.8%). These values might not be biologically significant. Solanas *et al*. (2008) showed that extrusion decreased crude protein Deg_{eff} at outflow rate 0.06/h by 8.8% (from 84.9% to 77.4%). Crude protein Deg_{eff} in this study was similar to findings of Cros *et al*. (1992) and Aufrère *et al*. (2001), which resulted in a RUP increase of 8.96%, allowing increasing metabolisable protein availability and consequently amino acid availability in the small intestine. Studies by Barchiesi *et al*. (2018) found a decrease in crude protein Deg_{eff} of 11.7% (from 82.3% to 72.7%) of lupins through extrusion at an outflow rate of 0.06/h by, with an increased true ileal crude protein digestibility. It is well established that effective protein degradability is negatively related to nitrogen digestibility (Zagorakis *et al*., 2015). This confirms that high protein degradability (high soluble fraction) negatively affects total tract protein digestibility.

Different results reported among studies may be due to different processing conditions (temperature, moisture and time exposed to these conditions) and the type and composition of feed

sample preparation (particle size), as well as legume species used (which is not always specified). Most studies were done on *L. albus* and information on *L. angustifolius* is scarce.

Although no differences in degradation rate were seen between lupin types in this study, it is important to look at the other nutrients in the two sources before purchasing or using it to formulate feed diets. There is variation in degradability within feeds (different varieties, processing methods and conditions) and care must be taken when using degradability figures from literature as some overlap is expected (Erasmus *et al.,* 1988).

This study clearly showed that the benefits of extrusion could be reached at a relatively lower temperature (116 ºC) than previously advised (130 ºC; Solanas *et al.,* 2008). The possibility for further improvement still exists by adjusting the processing conditions. According to literature the effect of processing can be strengthened by increasing the moisture applied and/or adding a source of reducing sugar (Hoskin & Dimick, 1995; Solanas *et al.,* 2005). High carbohydrate content or addition of sugars could also favour the development of the Maillard reaction and hence the possible favourable effect of extrusion.

3.6 Conclusion

Extrusion was found to modify ruminal degradation parameters of broad-leaf *L. albus* and narrow-leaf *L. angustifolius*, while also decreasing the effective rumen degradation of the two lupin types. Thereby, the RUP fraction of lupins is increased with a reduction in CP rumen degradability of up to 28% at a high outflow rate. The effect of extrusion of the two lupin types on protein degradability seems to be more pronounced at a faster outflow rate. This means that the use of lupins, which previously have been limited in diets for high producing animals due to its high RDP content, could be included in diets at higher levels following extrusion. It is recommended to also evaluate the extruded products in terms of amino acid availability in the small intestine. This should be done through bioassays to be able to generate true ileal digestibility values of crude protein to be used in feeds. Further studies of the extrusion conditions to determine optimum temperature and moisture of lupins during processing is needed in order to optimise the RUP fraction of lupin cultivars. Further studies are also needed to determine the effect of feeding extruded lupins on the digestion and growth or production performance of ruminants.

3.7 References

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Chapter 4

The effect of extrusion with molasses on the rumen undegradable protein fraction of canola oilcake meal and crushed sweet lupins

4.1 Abstract

The effect of extrusion of canola oilcake meal (CM) and crushed sweet lupins (CL) with molasses on the dry matter and crude protein degradability was determined *in situ*. Locally sourced CM and CL, with the addition of 6% molasses, were extruded with a Millbank extruder at a maximum temperature of 116 °C. Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula, were used in this trial. The sheep had *ad libitum* access to clean drinking water and a basal diet of wheat straw and lucerne hay (50:50) during the experimental period. Samples were incubated in the rumen of the sheep in polyester bags at intervals of 0, 2, 4, 8, 16, 24 and 48 hours. The dry matter (DM) and crude protein (CP) disappearances from the rumen were determined and then used to estimate the *in situ* DM and CP degradability parameters. Extrusion significantly lowered the CP soluble fraction of CM by 62.2%. The soluble fraction of CM did not differ from CL (46.0%) and CL did not differ significantly from crushed sweet lupins extruded (CLE, 38.2%). Extrusion increased the CP potential degradable fraction by 43.5%. The highest rate of degradation of the potential degradable fraction was found for CL (0.130%/h), which differed significantly from CLE (0.044%/h), CM (0.063%/h) and CME (0.053%/h). At each outflow rate, namely 0.02, 0.04, 0.05, 0.06 and 0.08/h, the CP effective degradability was lower for CM than for CL. The average effective degradability for CM was 68.2% and 78.0% for CL. Extrusion significantly lowered the CP effective degradability for both protein sources at every outflow rate tested. The biggest effect was seen at 0.08/h where effective degradation was lowered by 25.6% from 74.5% to 55.4%. Extrusion with molasses was found to modify ruminal degradation parameters of both canola oilcake meal and crushed sweet lupins, while also decreasing the effective rumen degradation, especially at faster outflow rates. Thereby, the rumen undegradable protein fraction was increased by 85.4%. This study showed that the benefits of extrusion can be reached at a relatively low temperature of 116 ºC and with the addition of 6% molasses. The possibility for further improvement still exists by adjusting the processing conditions. Achieved results should also be tested in a biological study to determine the availability of amino acids in the rumen undegradable protein fractions.

4.2 Introduction

Improving the productivity of livestock and meeting future demands for animal products and food security can be achieved if high producing animals are fed according to their specific nutrient

requirements (González *et al*., 2018). The increased demand for animal products leads to the strategic use of nutrition for enhancing production (Martin & Kadokwa, 2006). This increased demand for animal and plant protein products may lead to the availability of protein meals becoming limited or cost-prohibitive. In addition, geopolitical crises and weakening economies in particular regions may also limit or prevent trade. This could be especially detrimental in parts of the world where soybeans (the most popular plant protein sources in animal feeds) do not grow or where soybeans and soybean meals have to be imported. Therefore, it is important to have local feed ingredient alternatives and nearby processing capacity to contribute to increased feed security (Albin, 2015).

Canola oilcake meal is a by-product of oil production from canola seeds and is commonly incorporated in ruminant rations as a protein supplement due to its desirable amino acid profile (Newkirk *et al*., 2003; Santos, 2011). The protein content of canola meal differs depending on variety, growth conditions and oil extraction method, but it is accepted as having a high average crude protein content of 36-40% (Paula *et al*., 2018). Solvent and expeller oil extracted canola meal produced in South Africa contains 31.6% and 42.8% crude protein respectively on a dry matter basis (Brand *et al*., 2001). Lupins are widely used as a source of protein and energy in livestock feeds. Sweet lupins are largely free of anti-nutritional factors and have low risk of causing acidosis due to low starch levels and high fermentable carbohydrates (Dixon & Hosking, 1992). The relatively high crude protein content of lupins (34%, Brand *et al*., 2004) makes it a valuable resource for ruminant nutrition as they are also cost competitive. Lastly, lupins are nitrogen fixating and can be used in crop rotation systems with small cereals to reduce the need for inorganic nitrogen fertilizers (Abraham *et al*., 2019).

Canola oilcake meal and lupins can be used to partially replace soybean meal in ruminant diets, but inclusion is restricted due to high rumen degradable protein (RDP) contents of these ingredients (77% and 81%, respectively; NRC, 2001). Rumen degradable protein are soluble proteins that are degraded extensively in the rumen by microbes. This results in large scale ammonia production in ruminants which requires energy to be converted to urea in the liver; much of which is excreted through urine (McDonald, 1948). Only a small portion of urea is recycled in the rumen to contribute nitrogen that will be used to produce microbial protein. In order to increase the efficiency of protein utilisation from the highly degradable protein sources, these proteins need to be protected from excessive ruminal degradation, allowing the protein to bypass the rumen (rumen undegradable protein, RUP). The RUP then gets digested to amino acids, which will be absorbed from the small intestine of the ruminant and will be directly available for production (Walli, 2005; Makkar & Beever, 2013).

Highly degradable protein sources such as canola oilcake meal and lupins could possibly be extruded to decrease the rumen degradability thereof (to 54 and 67% respectively; INRA-CIRAD-AFZ Feed Tables, 2020) and increase the RUP fraction, before inclusion in ruminant diets. Extrusion is a heat treatment with added pressure and moisture that leads to the Maillard reaction and partial

protein denaturing that reduces the rumen degradability of protein sources without impairing protein digestibility in the small intestine, thus increasing the supply of RUP and intact amino acids to the small intestine. Processing conditions can alter the quality of protein. Van Soest (1987) suggested that the optimum heat input depends on many factors, namely moisture content, carbohydrate content and composition, protein content and presence of sulphate. Therefore optimum parameters of heat treatment may vary from one dietary protein source to another. Inclusion of heat-treated canola for dairy cows showed improved milk production due to higher RUP (Jones *et al.,* 2001; Wright *et al.,* 2005). Therefore, improving efficiency of feed conversion to meat or milk can have a significant impact on the profitability of a ruminant enterprise (Makkar & Beever, 2013).

Chalupa (1975) suggested that the Maillard reaction between sugar aldehyde groups and free amino groups can be controlled to decrease protein degradability in the rumen without adversely affecting intestinal protein digestibility. The treatment of soybean meal and canola oilcake meal with xylose was successful in decreasing the RDP fraction thereof (Cleale *et al*., 1987; Harstad & Prestløkken, 2000; Tuncer & Sacakli, 2003). Paula *et al*. (2017) added 2-3% molasses to canola meal before extrusion to increase the browning reaction.

The aim of this study was to determine the effect of a certain extrusion method with molasses on the *in situ* degradability of locally produced canola oilcake meal and crushed sweet lupins.

4.3 Materials and Methods

Animals and diets

Ethical clearance for this research was granted by the Animal Care and Use Research Ethics Committee of the University of Stellenbosch (Ethical clearance number #0378 and #0379). Six Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula, were housed in enclosed individual pens (1 m x 2 m) at the Welgevallen Experimental Farm of the University of Stellenbosch for the duration of the trial. The sheep had *ad libitum* access to clean drinking water and were supplied a basal diet of wheat straw and lucerne hay (50:50) *ad libitum* during the experimental period. The feed was replenished twice daily (every morning and evening) as necessary. The daily intake was estimated as 3% of the body weight of the sheep. The sheep were already adapted to the feed before the *in situ* trial started.

Treatments

Locally produced canola oilcake meal and crushed sweet lupins were sourced from SOILL, Moorreesburg (Western Cape, South Africa) and 6% molasses (Kalori 3000, Yara Animal Nutrition) were added in order to execute the experiments. Half of each batch was kept and the other half was extruded with temperatures reaching a maximum of 116 °C (at 13% moisture) with a Millbank extruder that is based on the Insta Pro 2000RC model (maximum capacity of 1 ton per hour, motor

at 55 kW and feeder motors 1.5 kW). The following four feeds were tested in this trial: canola oilcake meal not extruded (CM), canola oilcake meal extruded (CME), crushed sweet lupins not extruded (CL) and crushed sweet lupins extruded (CLE). These four feeds were used for further analysis after it was milled through a 2 mm size screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA).

In situ evaluation of ruminal degradability

The dry matter (DM) and crude protein (CP) degradabilities of CM, CME, CL and CLE were determined by the *in situ* technique described by Ørskov & McDonald (1979). The feed samples were dried in a force draught oven for a minimum of 48 hours at 60 °C, after which 5 g samples were weighed off on a digital scale and inserted into each of a series of previously dried, weighed and marked polyester bags (12 cm x 9 cm) with mean pore size of 15 µm. The bags were tied off by nylon strings of different lengths to prevent the strings from knotting in the rumen, as well as to ensure easy retrieval of the bags. Bags were incubated in the rumen at different time intervals, being 2, 4, 8, 16, 24 and 48 hours. An incubation series started when the first bag was inserted into the rumen cannula at 14h00 in the afternoon. The incubation was ended when all of the bags were removed at the same time after 48 hours. After bag removal, the bags were submerged in ice water to rapidly stop further degradation. The bags were washed under running tap water until water squeezed from it was clear. The 0 hour bag was prepared in the same way and was washed under the tap like the rest without being placed in the rumen. All bags were dried after incubation in a force draught oven for a minimum of 48 hours at 60 °C.

The treatments were randomly assigned to sheep during each period. This procedure was repeated in six periods as a completely randomised design, giving a total of six observations for each variable studied.

Chemical analysis

After drying the bags for 48 hours at 60 °C, the nylon strings were removed and the dried bags were weighed to determine the DM residue. The nitrogen content (%) of the residue was then determined using the Dumas combustion method (Method 990.03; AOAC, 2002) using a LECO TruMac N Nitrogen Determinator, version 1.3X (LECO Corporation, Michigan, USA). The CP content of the dry matter was determined by multiplying the percentage nitrogen by a factor of 6.25.

The chemical properties of CM, CME, CL and CLE before rumen incubation were determined with the official methods as described by the Association of Official Analytical Chemists (AOAC, 2002) for DM (method 934.01), ash (method 942.05), CP (method 990.03) and crude fat (method 2003.06). NDF and ADF were determined according to Van Soest *et al*. (1991), and calcium and phosphorus by method 6.1.1 of the Agri Laboratory Association of Southern Africa guidelines (ALASA, 1998).

Statistical analysis

Dry matter and CP disappearances were expressed as percentages of the amount remaining after rumen incubation. The percentage material degraded was fitted to the following one-compartment model, as proposed by Ørskov & McDonald (1979), by non-linear regression procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2014) to determine DM and CP degradability parameters:

$$\mathrm{Deg} = A + B\ (1 - \mathrm{e}^{-CT})$$

where Deg = actual degradation at time, *T (*%)

A = rapidly soluble fraction, intercept of degradation curve at T = 0 (%)

B = the fraction that will degrade over time, potential degradable fraction, or asymptote (%)

e = the base of natural logarithms

C = the rate constant for degradation of the *B* fraction (%/h)

Ruminal retention time affects the extent of degradation and therefore a fractional outflow rate of undegraded protein from the rumen (*k*) was taken into account when the percentage effective degradation (Deg_{eff}) was calculated as: $Deg_{eff} = A + BC/(C+k)$ at chosen values 0.02 (low intake level), 0.04, 0.05 (medium intake level), 0.06, 0.08/h (high intake level).

Non-linear parameters *A*, *B* and *C*, as well as the Deg_{eff} values, were submitted to a two-way analysis of variance (ANOVA) using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2014). Significance was declared at $P \leq 0.05$ and tendencies at $P < 0.10$ using Bonferroni tests.

4.4 Results

The chemical composition of the raw materials used in this study, both extruded and not extruded samples, is presented in Table 4.1. Extrusion did not seem to have a noticeable effect on the chemical composition of canola oilcake meal, with CP content of 33.6% for CM and 34.3% for CME. Extrusion seemed to possibly cause a slightly higher CP content for crushed sweet lupins (CL 26.6% and CLE 29.5%). The NDF content of extruded crushed sweet lupins seemed to be lower than unprocessed samples (18.9% and 22.14%, respectively).

Table 4.1 The chemical composition of canola oilcake meal and crushed sweet lupin seed samples which all had an addition of 6% molasses and were either extruded or unprocessed. All values (except DM) are expressed on a DM basis

Feed	DM (%)	Ash (%)	CP (%)	CF (%)	NDF (%)	ADF (%)	Ca (%)	P (%)
Canola oilcake meal	89.7	7.4	33.6	4.1	24.3	18.1	1.1	1.1
Canola oilcake meal extruded	89.3	8.1	34.3	3.6	23.4	16.9	1.0	1.1
Crushed sweet lupins	88.9	6.1	26.6	3.8	22.1	17.1	1.0	0.4
Crushed sweet lupins extruded	88.5	5.5	29.5	3.6	18.9	16.6	0.9	0.6

DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fibre, ADF = acid detergent fibre, Ca = calcium, P = phosphorus

The *in situ* DM degradability parameters showed interaction between the main effects of protein source and processing for the rapidly soluble fraction, but not for the potential degradable fraction, nor for the rate of degradation of *B*. Thus, the results are presented in Table 4.2 as main effects and with an interaction table. Canola oilcake meal presented the highest rapidly soluble fraction (42.5%) which differed significantly from CME (34.5%), CL (34.7%) and CLE (32.7%), which in turn did not differ from each other. Between protein sources, CM had a lower potential degradable fraction than CL (51.2% and 67.8% respectively). Extrusion increased the potential degradable fraction for DM from 53.6% to 65.4%. No differences were observed between protein sources for the rate of degradation of the degradable fraction, while extrusion did lower the rate of degradation from 0.071 to 0.046%/h.

Table 4.2 The effect of extrusion on the LS means (± SE) *in situ* dry matter rumen degradability non-linear parameters of canola oilcake meal and crushed sweet lupin seeds (6% molasses added to all treatments before processing)

		*Dry matter non-linear parameters		
		A	***B***	***C***
Protein source	Canola oilcake meal	38.5^{1} ± 0.7	51.2^{2} ± 2.0	0.058 ± 0.005
	Crushed sweet lupins	33.7^{2} ± 0.6	67.8^{1} ± 1.8	0.059 ± 0.004
	P-value	<0.001	<0.001	0.904
Processing	Not extruded	38.6^{1} ± 0.6	53.6^{2} ± 1.7	0.071^{1} ± 0.004
	Extruded	33.6^{2} ± 0.7	65.4^{1} ± 2.1	0.046^{2} ± 0.005
	P-value	<0.001	<0.001	0.002
Protein source x Processing	Canola oilcake meal	42.5^{a} ± 0.8	42.7^{b} ± 2.4	0.067^{ab} ± 0.01
	Canola oilcake meal extruded	34.5^{b} ± 1.0	59.8^{a} ± 3.2	0.050^{ab} ± 0.01
	Crushed sweet lupins	34.7^{b} ± 0.8	64.5^{a} ± 2.4	0.075^{a} ± 0.01
	Crushed sweet lupins extruded	32.7^{b} ± 0.9	71.0^{a} ± 2.6	0.043^{b} ± 0.01
	P-value	0.002	0.060	0.276

**A* = rapidly soluble fraction (%), *B* = the potential degradable fraction (%), *C* = the rate of degradation of the *B* fraction (%/h)
[1,2] Denote significant (*P* <0.05) differences in columns between protein source as well as processing
[a,b] Denote significant (*P* <0.05) differences in columns between treatments

The DM effective degradability showed interactions at each of the outflow rates, except for 0.08/h and for that reason it is presented in Table 4.3 as main effects of protein source and processing, as well as an interaction table. At an outflow rate of 0.08/h, canola oilcake meal was significantly lower (58.8%) than crushed lupins (61.4%). At outflow rates of 0.02, 0.04, 0.05 and 0.06/h, there were no significant differences between CM and CME but there were significant differences between CL and CLE where extrusion lowered the DM effective degradability, on average, from 76.4% to 55.2%.

Table 4.3 The effect of extrusion on the LS means (±SE) *in situ* dry matter effective degradation from the rumen at different outflow rates of canola oilcake meal and crushed sweet lupin seeds (6% molasses added to all treatments before processing)

		Dry matter effective degradation at fractional outflow rate (%)				
		0.02/h	**0.04/h**	**0.05/h**	**0.06/h**	**0.08/h**
***Protein source**	Canola oilcake meal	75.1^{2} ± 0.5	67.2^{2} ± 0.6	64.6^{2} ± 0.7	62.3^{2} ± 0.7	58.8^{2} ± 0.8
	Crushed sweet lupins	82.9^{1} ± 0.5	72.7^{1} ± 0.6	69.0^{1} ± 0.6	66.0^{1} ± 0.7	61.4^{1} ± 0.7
	P-value	<0.001	<0.001	<0.001	0.001	0.024
***Processing**	Not extruded	80.1^{1} ± 0.5	72.4^{1} ± 0.5	69.7^{1} ± 0.6	67.3^{1} ± 0.6	63.5^{1} ± 0.7
	Extruded	78.0^{2} ± 0.5	67.4^{2} ± 0.6	63.9^{2} ± 0.7	61.02 ± 0.8	56.7^{2} ± 0.8
	P-value	0.008	<0.001	<0.001	<0.001	<0.001
Protein source x Processing	Canola oilcake meal	74.7^{c} ± 0.6	68.2^{b} ± 0.7	66.3^{b} ± 0.8	64.4^{b} ± 0.9	61.4^{b} ± 0.9
	Canola oilcake meal extruded	75.5^{c} ± 0.8	66.1^{b} ± 1.0	62.9^{b} ± 1.1	60.3^{b} ± 1.2	56.3^{c} ± 1.2
	Crushed sweet lupins	85.4^{a} ± 0.6	76.5^{a} ± 0.7	73.2^{a} ± 0.8	70.3^{a} ± 0.9	65.7^{a} ± 0.9
	Crushed sweet lupins extruded	80.4^{b} ± 0.7	68.8^{b} ± 0.8	64.9^{b} ± 0.9	61.8^{b} ± 1.0	57.0^{c} ± 1.0
	P-value	<0.001	0.003	0.017	0.036	0.099

*n=12
^{1,2} Denote significant (*P* <0.05) differences in columns between protein source as well as processing
$^{a\text{-}c}$ Denote significant (*P* <0.05) differences in columns between treatments

The *in situ* crude protein degradability parameters are presented in Table 4.4 as main effects and an interaction table, as an interaction was observed for the rapidly soluble fraction as well as the rate of degradation for the potential degradable fraction but not for the potential degradable fraction. The rapidly soluble values of unprocessed CM (49.2%) and CL (46.0%) did not differ from each other (P >0.05). Extrusion was found to significantly lower the soluble fraction from 49.2% for CM to 18.6% for CME. On the other hand, the effect of extrusion had no significant impact on the soluble fraction of CL. No difference was found for the potential degradable fraction between protein source, while. extrusion increased (*P* <0.001) the potential degradability from 51.3% to 73.6%. The highest rate of degradation of the potential degradable fraction was found for CL (0.130%/h), which differed significantly from CLE (0.044%/h), CM (0.063%/h) and CME (0.053%/h). The expected CP disappearance from the rumen over time, derived from the *in situ* CP degradability parameter estimates, is presented graphically in Figure 4.1.

Table 4.4 The effect of extrusion on the LS means (± SE) *in situ* crude protein rumen degradability non-linear parameters of canola oilcake meal and crushed sweet lupin seeds (6% molasses added to all treatments before processing)

		*Crude protein non-linear parameters		
		A	***B***	***C***
Protein source	Canola oilcake meal	33.9^{2} ± 1.6	63.5 ± 3.4	0.058^{2} ± 0.01
	Crushed sweet lupins	42.1^{1} ± 1.4	61.5 ± 3.0	0.087^{1} ± 0.01
	P-value	<0.001	0.665	0.005
Processing	Not extruded	47.6^{1} ± 1.3	51.3^{2} ± 2.9	0.097^{1} ± 0.01
	Extruded	28.4^{2} ± 1.6	73.6^{1} ± 3.5	0.049^{2} ± 0.01
	P-value	<0.001	<0.001	<0.001
Protein source x Processing	Canola oilcake meal	49.2^{a} ± 1.9	48.4^{c} ± 4.1	0.063^{b} ± 0.01
	Canola oilcake meal extruded	18.6^{c} ± 2.5	78.5^{a} ± 5.4	0.053^{b} ± 0.01
	Crushed sweet lupins	46.0^{ab} ± 1.9	54.2^{bc} ± 4.1	0.130^{a} ± 0.01
	Crushed sweet lupins extruded	38.2^{b} ± 2.1	68.7^{ab} ± 4.4	0.044^{b} ± 0.01
	P-value	<0.001	0.102	<0.001

**A* = rapidly soluble fraction (%), *B* = the potential degradable fraction (%), *C* = the rate of degradation of the *B* fraction (%/h)
[1,2] Denote significant (*P* <0.05) differences in columns between protein source as well as processing
[a-c] Denote significant (*P* <0.05) differences in columns between treatments

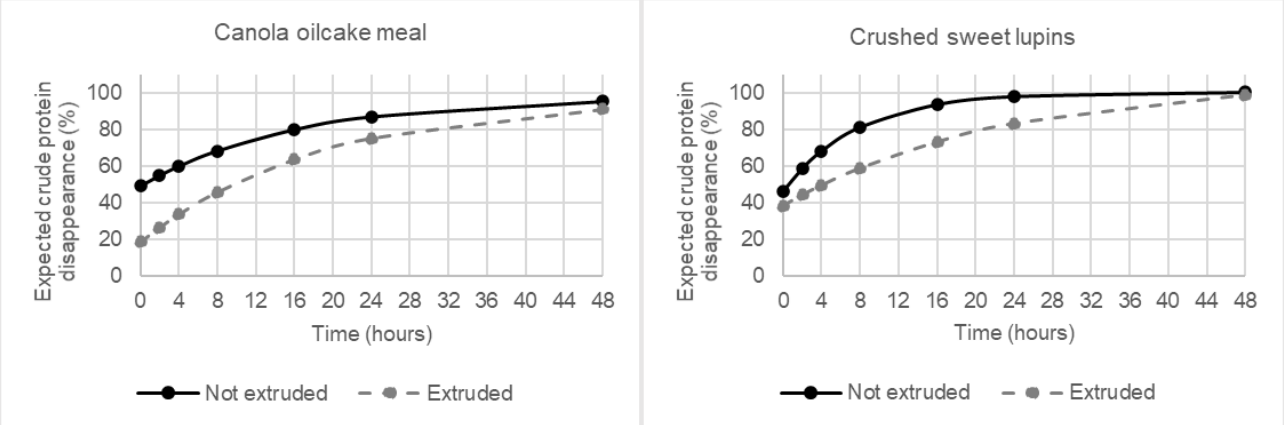

Figure 4.1 Expected crude protein disappearance from the rumen over time for canola oilcake meal and crushed sweet lupins, extruded or unprocessed (6% molasses added to all treatments before processing).

No interactions were observed between the main effects for crude protein effective degradability and thus the main effects are presented in Table 4.5. At each outflow rate, 0.02, 0.04, 0.05, 0.06 and 0.08/h, the effective degradability was lower (*P* <0.001) for canola oilcake meal than for crushed sweet lupins. The average effective crude protein degradability for canola meal was 68.2% and 78.0% for crushed lupins. Extrusion significantly lowered the CP effective degradability at every tested outflow rate. The biggest effect was seen at an outflow rate of 0.08/h, where effective degradation was lowered (*P* <0.001) by 25.6%, from 74.5% to 55.4%. This is expected as 0.08/h is the outflow rate representing high producing animals at a faster outflow rate of digesta from the rumen.

Table 4.5 The effect of extrusion on the LS means (± SE) *in situ* crude protein effective degradation from the rumen at different outflow rates of canola oilcake meal and crushed sweet lupin seeds (6% molasses added to all treatments before processing)

		*Crude protein effective degradation at fractional outflow rate (%)				
		0.02/h	**0.04/h**	**0.05/h**	**0.06/h**	**0.08/h**
Protein source	Canola oilcake meal	80.0^{2} ± 0.8	70.3^{2} ± 0.8	66.8^{2} ± 0.9	64.0^{2} ± 0.9	59.6^{2} ± 1.0
	Crushed sweet lupins	88.3^{1} ± 0.7	79.9^{1} ± 0.7	76.9^{1} ± 0.8	74.4^{1} ± 0.8	70.4^{1} ± 0.9
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
Processing	Not extruded	88.9^{1} ± 0.7	82.5^{1} ± 0.7	80.0^{1} ± 0.7	78.0^{1} ± 0.8	74.5^{1} ± 0.9
	Extruded	79.5^{2} ± 0.8	67.7^{2} ± 0.8	63.7^{2} ± 0.9	60.4^{2} ± 1.0	55.4^{2} ± 1.0
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001

*n=12
[1,2] Denote significant (*P* <0.05) differences in columns between protein source as well as processing

4.5 Discussion

Regarding the nutrient compositions of the feeds analysed, CLE showed a slightly lower NDF content than that of the unprocessed crushed lupins (18.9% and 22.1% respectively). This could be as a result of the extrusion process due to partial depolymerization of polysaccharides of the cell wall, which makes it more soluble in the acid and alkali solutions used during measurements (Solanas *et al.,* 2005). This has been observed by other authors using extruded lupins (Cros *et al*., 1992; Barchiesi-Ferrari & Anrique, 2011). The CP content of CLE seemed slightly higher than unprocessed lupins (26.6% and 29.5%), which may be due to the release of peptides from cell walls during the extrusion process. The crude protein value of sweet lupins in this study (26.6% unprocessed and 29.5% extruded) was lower than the value of 35.5% used by Brand *et al*. (1992). One possible reason could be the addition of molasses in this study, which would also explain the lower DM of lupins in this study as molasses (Kalori 3000, Yara Animal Nutrition) has a high hygroscopicity.

The reason for the observed interaction in the DM soluble fraction could be due to extrusion lowering the soluble fraction for CM while this was not observed for CL. Dry matter effective degradability showed interaction at every outflow rate except 0.08%/h. This could be due to no difference being observed between CM and CME while decreases were observed for CLE compared to CL at outflow rates of 0.02, 0.04, 0.05 and 0.06%/h. The DM effective degradation at 0.08%/h for canola oilcake meal was lower than crushed lupins, namely 58.8% and 61.4% respectively, and extrusion decreased the DM effective degradation at 0.08%/h by 10.7%.

The objective for decreasing the RDP fraction of protein sources by extrusion is to minimise the rapidly soluble fraction and maximise the slowly or potential degradable fraction with little or no increase in the indigestible fraction, which have not been covered in this study (Van Soest, 1987).

Extrusion at 116 ºC in this study significantly lowered the CP rapidly soluble fraction of canola oilcake meal by 62%. Similar results for canola meal heated at 125 ºC was found by Mustafa *et al*.

(1997) and Griffiths (2004), who extruded canola meal at 120 ºC. Michalak & Potkanski (2005) showed that extrusion at 140 ºC of rapeseed oilmeal lowered the soluble fraction by 13%. Barchiesi-Ferrari & Anrique (2011) extruded rapeseed meal at 120 ºC which decreased the rapidly soluble fraction by 13.5%. No significant effect was seen with crushed lupins for the soluble fraction, but in Chapter 3 with extruded lupins at 116 ºC, the rapidly soluble fraction for lupins was lowered by an average of 48.4%. Kibelolaud *et al*. (1993) extruded white *L. albus* seeds and reported a 4% increase in the soluble fraction when extruded at 110 ºC, but a 19.3% decrease at 130 ºC. Griffiths (2004) extruded lupins at 120 ºC and found a 6% increase in the rapidly soluble fraction. Solanas *et al*. (2008) extruded *L. albus* at 140 ºC, which resulted in no difference in the rapidly soluble fraction (35% and 34.5%). Barchiesi-Ferrari & Anrique (2011) extruded dehulled *L. albus* at 130 ºC (20% moisture), which decreased the soluble fraction by 12.4% (from 42.7 to 37.4%). Barchiesi *et al*. (2018) extruded dehulled *L. albus* at 140 ºC (20% moisture), which led to a decrease of 29% in the soluble fraction.

In this study extrusion increased the CP potential degradable fraction by 30.3% while no effect was seen between protein source. Kibelolaud *et al*. (1993) found a 18.6% decrease in the potential degradable fraction for lupins extruded at 110 ºC. However, a 43.1% increase was seen for extrusion at 130 ºC, which indicates that a higher temperature showed better results for extrusion in that particular study. Solanas *et al*. (2008) extruded *L. albus* at 140 ºC, which resulted in no difference in the potential degradable fraction (62.1% and 64.2%), while Barchiesi *et al*. (2018) found a 4% increase at the same temperature. Barchiesi-Ferrari & Anrique (2011) extruded dehulled *L. albus* at 130 ºC, which led to a 4.7% increase in the potential degradable fraction. Mustafa *et al*. (1997) heated canola meal at 125 ºC and found an 18.9% increase in the potential degradable fraction. Michalak & Potkanski (2005) showed that extrusion at 140 ºC of rapeseed oilmeal increased the potential degradable fraction by only 4.9%. Barchiesi-Ferrari & Anrique (2011) extruded rapeseed meal at 120 ºC (20% moisture) and found no difference in the potential degradable fraction (45.1% and 45.7%).

Extrusion did not affect the rate of degradation of the potential degradable fraction for canola oilcake meal in this study. Barchiesi-Ferrari & Anrique (2011) also found no difference when extruding rapeseed meal at 120 ºC. However, Mustafa *et al*. (1997) heated canola meal at 125 ºC for 20 min and found a decrease in the rate of degradation from 6%/h to 1.4%/h. The rate of degradation lowered significantly with 66.2% for crushed sweet lupins in this study. The same trend was seen in lupins from Chapter 3 of this study and by other authors (Kibelolaud *et al*., 1993; Griffiths, 2004; Solanas *et al*., 2008). Barchiesi *et al*. (2018) extruded dehulled *L. albus* at 140 ºC (20% moisture), which led to no difference in the rate of degradation (0.17% and 0.15%).

Canola oilcake meal showed lower CP effective degradability at every outflow rate tested compared to crushed sweet lupins (respective averages, 68.1% and 78%). Extrusion significantly lowered the CP effective degradability at every outflow rate tested, 0.02, 0.04, 0.05, 0.06 and

0.08%/h, by 10.6%, 18%, 20.3%, 22.6% and 25.6%, respectively. The CP effective degradation of extruded lupins in this study is lower than the observed degradabilities of lupins in Chapter 3, which might be due to the addition of molasses which promotes the Maillard reaction and lowers rumen degradability. The effect could also be seen in lower CP rapidly soluble fractions and rate of degradation, as well as higher potential degradable fraction values, in this study. Previous studies found that heating rapeseed at high temperatures (150 ºC and 200 ºC, Lindberg, 1982) and lupins (195 ºC, Benchaar & Moncoulon, 1993) decreased effective degradability while no effect was found at 100 ºC by Lindberg (1982). Kibelolaud *et al*. (1993) extruded white *L. albus* seeds at 110 ºC and 130 ºC, which decreased the CP effective degradation at the 0.06%/h outflow rate by 3.9% and 14.5%, respectively. In contrast, McKinnon *et al*. (1995) found that heating canola meal to 145 ºC for 30 min decreased intestinal and total tract digestibility. However, heating at 125 ºC lowered ruminal degradability without negative effects on intestinal digestibility. Similarly, Michalak & Potkanski (2005) showed that extrusion at 140 ºC of rapeseed oilmeal reduced effective degradability by 30% and shifted protein digestion from the rumen to the small intestine, giving similar total tract protein digestibility. This shows that higher temperatures are not necessarily needed to achieve the benefits of extrusion and heat treatment. High temperatures could possibly lead to heat damage and increased production costs.

Other authors that tested heat treated lupins found decreased CP ruminal degradability at 120 ºC and 150 ºC (by 1.7% and 25.2%, respectively at 16 hours, Cros *et al*., 1992), 120 ºC (by 10.8% at outflow rate of 0.08/h, Griffiths, 2004), 140 ºC (by 19.4% at 12 hours, Solanas *et al*., 2005, by 8.8% at 0.06/h outflow rate, Solanas *et al*., 2008, by 11.6% at 0.06/h, Barchiesi *et al*., 2018), and 130 ºC (by 2,8% at 0.08/h outflow rate, Barchiesi-Ferrari & Anrique, 2011). Other studies with heat treated canola oilcake meal and rapeseed found decreases in CP ruminal degradability at 125 ºC (by 50%, Mustafa *et al*., 1997), 120 ºC (17.6% at 0.08/h outflow rate, Griffiths, 2004), and 120 ºC (by 10.5% at 0.08/h outflow rate, Barchiesi-Ferrari & Anrique, 2011). Paula *et al*. (2017) extruded canola meal with 2-3% added molasses and fed it to lactating dairy cows with no effect on milk yield and milk components. It did, however, decrease urinary nitrogen percentage, faecal nitrogen and milk urea concentration, thus may reduce environmental impact. An increase in nitrogen utilisation was also seen by Huhtanen *et al*. (2011) and Broderick *et al*. (2015).

Differences between studies could be due to intrinsic differences in the feeds or extrinsic differences such as smaller particle size and different fistulated animals (steers vs sheep), basal diets, *in situ* techniques, and mostly due to processing conditions (Habib *et al*., 2013). Van Soest (1987) suggested that the non-protein nitrogen and rapidly degradable true protein fractions denature at lower temperatures and become intermediately or slowly degradable, while the slowly degradable protein fraction responds at higher temperatures and usually becomes unavailable due to heat damage from the Maillard reaction. Optimum temperature and conditions vary from one dietary protein to another.

Extrusion with molasses at 116 ºC more than doubled the RUP fraction (calculated from the CP content on a dry matter basis and the effective degradability at 0.08%/h outflow rate) of the protein sources tested, which resulted in a more favourable ratio of RDP:RUP for inclusion in ruminant diets. The RDP fraction was decreased by increasing the RUP fraction of canola oilcake meal by 70.1% and crushed sweet lupins by 107.8% at an outflow rate of 0.08%/h. The intestinal degradability of these protein sources was not covered in this study.

4.6 Conclusion

Extrusion with molasses was found to modify ruminal degradation parameters of canola oilcake meal and crushed sweet lupins, while also decreasing the effective rumen degradation, especially at faster outflow rates. Thereby, the combined RUP fraction of canola oilcake meal and crushed sweet lupins has been increased by 85.4% through extrusion with molasses compared to not extruded (from 3.3% to 6.5%). This study showed that the benefits of extrusion could be reached by a relatively low temperature of 116 ºC with the addition of 6% molasses.

Regionally grown and properly processed feed materials, such as those produced from canola and lupins, play important roles in supporting the livestock production sector, which is the major and reliable source of animal proteins in human diets. Having some local processing of canola oilcakes and lupins with extrusion available will increase the use of local feeds in supporting and enhancing animal performance. Most importantly, it will make people less dependent on imports of raw materials and feed ingredients, which are in demand more and more around the world.

Further research is required to examine the effect of extrusion with molasses of canola oilcake meal and crushed sweet lupins on the intestinal digestibility, particularly its effect on the availability of essential amino acids.

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Chapter 5
The effect of extrusion with molasses and addition of chitosan on the rumen undegradable protein fraction of soybean oilcake meal

5.1 Abstract

The process of extrusion and the addition of a polymer, chitosan, have shown potential to reduce the rumen degradability of soybean oilcake meal to increase the nutritional value thereof to ruminants. The aim of this study was to evaluate whether cold extrusion and the addition of chitosan and molasses would affect the rumen undegradable protein fraction of soybean oilcake meal. Soybean oilcake meal was mixed with 6% molasses before the addition of chitosan and extrusion. The chitosan solution of 1% was added to the soybean oilcake meal. Cold extrusion was performed at 40-45 °C with a custom made cold, single screw extruder. Four Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula, were used in this trial. Samples were incubated in the rumen of the sheep in polyester bags at intervals of 0, 2, 4, 8, 16, 24 and 48 hours. The *in situ* study was carried out as a cross-over design with four treatments and four sheep over four periods. The actual rumen disappearance values were compared at each incubation time point. This research showed no differences with cold extrusion or the addition of chitosan and molasses on the rumen undegradable protein fraction of soybean oilcake meal. The benefits of extrusion could not be reached with soybean oilcake meal and cold extrusion as applied in this study. Literature shows that chitosan has great potential as a feed additive by binding protein, but more research is needed to fully understand the mode of action of chitosan in the rumen and the bioavailability of bound protein in the small intestine.

5.2 Introduction

Animal nutritionists are continuously researching natural products that could enhance animal production. The efficiency and profitability of animal production can be optimised by improving animal performance (Haraki *et al*., 2018). Different means of increasing animal performance could include, but is not limited to, improved feed digestion, improving the feed conversion ratio and increasing the dietary nutrient density of feed, but, this could be difficult with increasing feed costs (Haraki *et al*., 2018). Plant protein sources make up the second largest proportion of livestock diets, of which soybean oilcake meal is the most common plant protein source used because of its high protein content and favourable amino acid composition (Tona, 2018). The potential exists to further process soybean oilcake meal to reduce its rumen degradability even further for an improved quality protein source to ruminants.

The true value of protein in ruminant diets depends largely on the extent to which feed proteins escape degradation in the rumen (also called bypass protein or rumen undegradable protein, RUP) and on their essential amino acid profile (Goiri *et al*., 2009c). Dietary protein is mostly degraded in the rumen (called rumen degradable protein, RDP) by microorganisms and digestive enzymes present in the rumen, which provides microbial protein to the small intestine (Annonier *et al*., 2001; Chiang *et al*., 2009). For protein to be optimally utilisable by the ruminant animal, a portion of dietary protein must pass through the rumen without degradation and only be digested in or after the abomasum so that amino acids can reach the absorption sites in the small intestine (especially duodenum) and be utilised (Annonier *et al*., 2001).

A good source of RUP should be able to stay stable in the rumen, in which the pH is in the range of 5.5 to 7.0, for an extended period and which permits quick release within a short period in the abomasum and small intestine, where the pH drops to 3.5. It is, however, important to note that the pH and rate of passage depend on different factors such as diet (Church, 1979; Annonier *et al*., 2001). Fadel El-Seed *et al*. (2003) attempted to use chitosan as a nitrogen source for rumen microbes as it contained 6.7% nitrogen, but found that chitosan is not degraded in the rumen and therefore suggested that chitosan could perhaps be used as a RUP source for ruminants. Theoretically, the characteristics of chitosan could make it a good source of RUP as chitosan is known to be readily soluble at pH below 6, depending on the degree of deacetylation (Rinaudo, 2006), and as the pH increases above 6 the polymer loses charge and becomes insoluble (Pillai *et al*., 2009).

Chitosan is referred to as a family of compounds which differ in molecular weight and degree of deacetylation rather than a single compound (Terbojevich *et al*., 1993). Chitosan is a non-toxic, biodegradable biopolymer. It is derived from deacetylated chitin found in the exoskeletons of insects, crustaceans and molluscs, as well as in the cell walls of fungi and certain algae (Li *et al*., 2018). It is the second most abundant polysaccharide in nature after cellulose (Li *et al*., 2018). Chitosan has received attention for its diverse potential application in medicine, food and cosmetics and is seen as a new feed additive in ruminant diets, primarily because of its antimicrobial activity (Del Valle *et al*., 2017). Chitosan is available or can be made in a range of different molecular weights and degree of acetylation (Terbojevich *et al*., 1993). Thus, the different physicochemical characteristics thereof result in different antimicrobial activities and chemical properties (Mima *et al*.,1983; Rhoades & Roller, 2000).

Ruminant nutrition studies using chitosan have given variable results. However, several studies have shown to change ruminal fermentation by shifting volatile fatty acid profiles, including higher propionate concentration and lower acetate to propionate ratio, which likely improve the energy efficiency of ruminal fermentation and reduced methane production in ruminants (Goiri *et al*., 2009a,b, 2010; Haryati *et al*., 2019; Seankamsorn *et al*., 2020). Mingoti *et al*. (2016) found a

reduction in nitrogen faecal excretion with chitosan supplementation, which might be related to improvement in protein digestibility.

Although studies have evaluated the effect of chitosan on ruminal fermentation, few studies have evaluated the effect of dietary chitosan inclusion on the rumen degradability parameters through *in situ* trials and specifically the combination of extrusion and addition of chitosan and the effect on soybean oilcake meal with added molasses.

Extrusion is a process in feed manufacturing where heat and pressure are applied in the presence of moisture, most commonly used for oil extraction, but also more recently to decrease rumen degradability of protein sources (White *et al*., 2007; Zagorakis *et al*., 2015). The raw material is fed through a barrel with increasing pressure as the barrel tapers toward the outlet. This method of protecting proteins is considered safe and economical. Extrusion causes denaturing of proteins, which decreases protein solubility and thus also decrease the ruminal degradability of protein in feeds (Barchiesi-Ferrari & Anrique, 2011). The RUP fraction is thus increased, providing greater quantities of amino acids available for absorption (Solanas *et al*., 2008). Cold extrusion was chosen for this study to prevent potential heat damage to already extruded soybean oilcake meal, and chitosan is also known to be sensitive to heat. Furthermore, information on the effect of cold extrusion on the rumen degradability of protein sources is scarce in literature.

Therefore, the aim of this study was to determine the effect of cold extrusion and the addition of chitosan on the *in situ* rumen degradability of soybean oilcake meal with added molasses.

5.3 Materials and Methods

Animals and diets

Ethical clearance for this research was granted by the Research Ethics Committee for Animal Care and Use of the University of Stellenbosch (Ethical clearance numbers #0378 and #0379). Four Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula, were housed in enclosed individual pens (1 m x 2 m) at the Welgevallen Experimental Farm of the University of Stellenbosch. The sheep had *ad libitum* access to clean drinking water and were supplied a basal diet of wheat straw and lucerne hay (50:50) *ad libitum* during the experimental period. The feed was filled twice daily (every morning and evening) as necessary. Daily intake was estimated as 3% of the body weight of the sheep. The sheep were already adapted to the feed before the *in situ* trial started.

Treatments

Soybean oilcake meal was thoroughly mixed with 6% molasses (Kalori 3000, Yara Animal Nutrition). Chitosan was sourced from Wellable Group Marine Biological & Chemical Co., Ltd. The chitosan had the following technical specifications: molar mass of 161 g/mol, bulk density of 0.35 g/ml, ash content of 1.36%, pH of 7.9, viscosity of <100 cPs, deacetylated degree of 95.8%. The

chitosan solution was prepared by dissolving 1% chitosan in a 1% glacial acetic acid solution with water using a heated magnetic stirrer. One portion of the soybean oilcake meal with molasses was kept as a control and another portion was thoroughly hand mixed with the chitosan solution. Both soybean oilcake meal treatments were dried in a force draught oven overnight at 50 °C. Half of the soybean oilcake meal with molasses and half of the soybean oilcake meal with molasses and chitosan was milled through a 2 mm Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) before being cold-extruded at 40-45 °C with a custom made cold, single screw extruder (Nutritionhub Pty Ltd, Stellenbosch, South Africa). After extrusion, the treatments were dried at 60 ºC for 4 hours to reach a 3% moisture content.

The four feeds tested in the trial were soybean oilcake meal with molasses not extruded, without chitosan (SOM), soybean oilcake meal with molasses extruded, without chitosan (SOME), soybean oilcake meal with molasses and chitosan, not extruded (SOMC) and soybean oilcake meal with molasses and chitosan and also extruded (SOMCE). All four feeds were milled through a 2 mm screen size using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) for the *in situ* analyses, according to NRC recommendations (NRC, 2001).

In situ evaluation of ruminal degradability

The dry matter (DM) and crude protein (CP) degradabilities of SOM, SOME, SOMC and SOMCE were determined by the *in situ* technique described by Ørskov & McDonald (1979). The feed samples were dried in a force draught oven for a minimum of 48 hours at 60 °C, after which 5 g samples were weighed off on a digital scale and inserted into each of a series of previously dried, weighed and marked polyester bags (12 cm x 9 cm) with mean pore size of 15 µm. The bags were tied off by nylon strings of different lengths to prevent the strings from knotting in the rumen, as well as to ensure easy retrieval of the bags. Bags were incubated in the rumen at different time intervals, being 2, 4, 8, 16, 24 and 48 hours, with an all-out approach. An incubation series started when the first bag was inserted into the rumen cannula at 14h00 in the afternoon. The following bags were inserted at each following incubation times. The incubation was ended when all the bags were removed at the same time after 48 hours. After bag removal, the bags were submerged in ice water to rapidly stop further microbial degradation. The bags were washed under running tap water until water squeezed from it was clear. The 0h bag was prepared in the same way and was washed under the tap until clear like the rest without being placed in the rumen. All bags were dried after incubation in a force draught oven for a minimum of 48 hours at 60 °C.

This procedure was carried out in a cross-over design where each of the four feeds was tested in each of the four sheep over four periods (one feed per sheep per period), giving a total of four observations for each variable studied.

Chemical analysis

After drying the bags for 48 hours at 60 °C, the nylon strings were removed and the dried bags were weighed to determine the DM residue. The nitrogen content (%) of the residue was then determined using the Dumas combustion method (Method 990.03; AOAC, 2002) using a LECO TruMac N Nitrogen Determinator, version 1.3X (LECO Corporation, Michigan, USA). The CP content of the dry matter was determined by multiplying the percentage nitrogen by a factor of 6.25.

The chemical properties of SOM, SOME, SOMC and SOMCE before rumen incubation were determined with the official methods as described by the Association of Official Analytical Chemists (AOAC, 2002) for DM (method 934.01), ash (method 942.05), CP (method 990.03) and crude fat (method 2003.06). NDF and ADF were determined according to Van Soest *et al*. (1991), and calcium and phosphorus by method 6.1.1 of the Agri Laboratory Association of Southern Africa guidelines (ALASA, 1998).

Statistical analysis

Dry matter and CP disappearances were expressed as percentages of the amount of treatment remaining in the bag after rumen incubation. The percentage material degraded did not fit the non-linear regression one-compartment model, as proposed by Ørskov & McDonald (1979). This might have been due to particle size variation between treatments or samples, as extrusion and milling of already extruded oilcake meals could have led to very fine particle sizes and thus an asymptote has not been reached within the 48 hour incubation period. The percentage particle distribution was determined by calculating the fineness modulus (ASTM C136 / C136M – 19, 2019) and can be seen in Table 5.1. Five gram samples of each of the treatments were manually put through the stack of sieves (Labotec sieves with sizes 1.18 mm, 850 µm, 250 µm, 180 µm, 150 µm, 125 µm, 106 µm and <106 µm) three times and the average was used to calculate the fineness modulus.

For this reason, the actual disappearance values obtained at each timepoint was submitted to an ANOCOVA with the 0h value as the covariate in order to adjust for the starting value of each treatment as interaction was found at 0h (representing the readily soluble particles and particles that was small enough to escape through the pores of the bag), using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2014). Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$ using Bonferroni tests.

5.4 Results

The fineness modulus of SOM, SOME, SOMC and SOMCE is presented in Table 5.1. Extrusion led to a bigger proportion of finer particles as the proportion left on the pan (<106 µm) was 5.0% and 10.3% for SOME and SOMCE compared to 2.5% and 2.4% for SOM and SOMC, respectively. Extrusion also slightly increased the proportion large particles left on the 1.18 mm sieve,

which were 2.6% and 2.2% for SOME and SOMCE compared to 0.4% and 0.6% for SOM and SOMC. Furthermore, the three treated feeds had more particles left on the 850 µm sieve: 29.0%, 21.2% and 21.9% for SOME, SOMC and SOMCE respectively compared to 14.5% for SOM. It therefore seems as if the extrusion process led to an increased proportion of bigger as well as finer particles.

Table 5.1 The fineness modulus of soybean oilcake meal (SOM), soybean oilcake meal extruded (SOME), soybean oilcake meal with chitosan (SOMC) and soybean oilcake meal with chitosan and extruded (SOMCE) (all treatments with addition of 6% molasses)

	Cumulative percentage retained on each sieve			
Sieve size	SOM	SOME	SOMC	SOMCE
1.18 mm	0.4	2.6	0.6	2.2
850 µm	14.9	31.6	21.8	24.1
250 µm	93.5	90.0	93.0	79.9
180 µm	95.9	92.8	95.4	84.7
150 µm	96.7	93.6	96.4	86.7
125 µm	97.0	94.1	96.9	87.7
106 µm	97.5	95.0	97.6	89.7
<106 µm	100.0	100.0	100.0	100.0
Fineness modulus	6.0	6.0	6.0	5.6

The chemical composition of the treatments used in this study, is presented in Table 5.2. It seems that the extrusion process increased the dry matter content of the soybean oilcake meal. Further, it seems that extrusion and the addition of chitosan, as well as the combination of both, increased the crude protein content of treatments compared to soybean oilcake meal with no processing and no polymer added.

Table 5.2 The chemical composition of soybean oilcake meal with an addition of 6% molasses either with or without 1% chitosan added and either extruded or unprocessed. All values (except DM) are expressed on a DM basis

Feed	**DM (%)**	**Ash (%)**	**CP (%)**	**CF (%)**	**NDF (%)**	**ADF (%)**	**Ca (%)**	**P (%)**
Soybean oilcake meal	86.3	7.9	43.8	0.8	6.9	4.6	0.7	0.7
Soybean oilcake meal extruded	95.9	8.5	47.6	0.6	9.0	5.7	0.8	0.8
Soybean oilcake meal with chitosan	89.7	8.0	46.0	0.6	8.7	4.9	0.8	0.7
Soybean oilcake meal with chitosan extruded	95.4	8.5	47.3	0.9	8.4	5.1	0.9	0.8

DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fibre, ADF = acid detergent fibre, Ca = calcium, P = phosphorus

The actual dry matter disappearance values at the different incubation times in the rumen is presented in Table 5.3. No interaction was observed between processing (extrusion) and polymer

(addition of chitosan), thus, the main effects could be interpreted separately. No differences were observed between the main effects for dry matter disappearance from the rumen.

Table 5.3 The effect of extrusion and addition of chitosan on the actual *in situ* dry matter rumen disappearance values (%) at 2, 4, 8, 16, 24 and 48 hours rumen incubation time of soybean oilcake meal with molasses

		*Actual dry matter disappearance values at incubation time (%)					
		2h	**4h**	**8h**	**16h**	**24h**	**48h**
Processing	Without extrusion	36.0 ± 0.5	38.9 ± 1.7	45.3 ± 4.0	52.8 ± 6.5	72.6 ± 8.7	93.2 ± 4.0
	With extrusion	36.7 ± 0.5	39.7 ± 1.7	53.7 ± 4.0	67.3 ± 6.5	81.9 ± 8.7	97.0 ± 4.0
	P-value	0.457	0.816	0.291	0.262	0.582	0.627
Polymer	Without chitosan	36.5 ± 0.4	40.2 ± 1.4	50.6 ± 3.2	65.7 ± 5.2	77.5 ± 7.0	96.1 ± 3.2
	With chitosan	36.3 ± 0.4	38.4 ± 1.4	48.4 ± 3.2	54.4 ± 5.2	77.1 ± 7.0	94.1 ± 3.2
	P-value	0.797	0.48	0.71	0.261	0.979	0.747

*Least square mean ± standard error, n=8
The 0 hour disappearance value was used as a covariate

The actual crude protein disappearance values at the different incubation times in the rumen are presented in Table 5.4. Again, no interaction was observed between processing (extrusion) and polymer (addition of chitosan), thus, the main effects could be interpreted separately. No differences were observed between main effects for crude protein disappearance from the rumen.

Table 5.4 The effect of extrusion and addition of chitosan on the actual *in situ* crude protein rumen disappearance values (%) at 2, 4, 8, 16, 24 and 48 hours rumen incubation time of soybean oilcake meal with molasses

		*Actual crude protein disappearance values at incubation time (%)					
		2h	**4h**	**8h**	**16h**	**24h**	**48h**
Processing	Without extrusion	21.6 ± 0.9	21.6 ± 1.6	30.2 ± 3.1	35.9 ± 6.8	65.7 ± 9.4	92.7 ± 4.3
	With extrusion	21.9 ± 0.9	24.6 ± 1.6	32.6 ± 3.1	46.3 ± 6.8	66.5 ± 9.4	95.6 ± 5.5
	P-value	0.858	0.317	0.677	0.407	0.962	0.743
Polymer	Without chitosan	22.0 ± 0.8	25.3 ± 1.4	29.9 ± 2.8	45.3 ± 6.1	60.6 ± 8.5	93.8 ± 5.0
	With chitosan	21.5 ± 0.8	20.9 ± 1.4	32.9 ± 2.8	36.8 ± 6.1	71.7 ± 8.5	94.5 ± 3.9
	P-value	0.703	0.108	0.551	0.439	0.468	0.928

*Least square mean ± standard error, n=8
The 0 hour disappearance value was used as a covariate

5.5 Discussion

With regards to the chemical composition of the treatments, a higher dry matter content for extruded feeds was expected, as it was dried to a moisture content of 3% as part of the extrusion process. Chitosan is known to have a relatively high nitrogen content of 6.9% (Ravi Kumar, 2000), which could explain the seemingly higher crude protein content of treatments containing chitosan in this study. No differences were observed for processing (extrusion) nor polymer (addition of chitosan) between treatments for dry matter or crude protein disappearances. It is unclear as to why

these results were found as it was expected to observe even slight changes in rumen degradability, especially with the inclusion of chitosan. Different aspects were incorporated into this study, including the *in situ* method, soybean oilcake meal, cold extrusion, chitosan, and molasses, which could have influenced the results.

The *in situ* method is an accepted method to evaluate rumen degradability, but it is not without shortcomings. It is known to overestimate the degradability of feeds, because of small particles that escape through the bag pores. The results of this study, however, showed lower values of disappearance than expected, especially from 2 to 16 hours incubation. Some of the factors that have the most influence on the *in situ* method were kept constant throughout the trial: bag characteristics (pore and bag size), the animals used (species and diet) and to a lesser extent the environment (Michalet-Doreau & Cerneau, 1991). Another characteristic that could have a major influence on the *in situ* method results, which could not be controlled beyond methodology, is the particle size of samples from the different treatments. The fineness modulus of samples presented in Table 5.1 shows that the extrusion process as used in this study could have led to an increased proportion of bigger and finer particles of the soybean oilcake meal. It can be expected that there will be considerable differences in particle size between the same feeds ground with the same screen. It has, therefore, been suggested that degradability studies should instead be based on particle size rather than the screen size of milling (Michalet-Doreau & Cerneau, 1991). Nevertheless, it is evident that the particle distribution differed between feeds tested in this study and this justifies the use of the 0 hour value as covariate as this corrects for the differences in fine particles and potentially the solubility thereof, as the other incubation times disappearance values is dependent on the 0 hour values. The grinding of the treatments before being used in *in situ* studies is inevitable and is necessary to reduce the degree of variation and to simulate mastication of feed, which is normal before digestion (Michalet-Doreau & Cerneau, 1991). It is accepted that a small proportion of the rumen degradability will increase with decreased particle size, but the increases observed vary between studies (Michalet-Doreau & Cerneau, 1991). Alternatively, smaller particle size has a greater surface to mass ratio, which is more accessible to microbes in the rumen, thus small particles could lead to an increase in microbial contamination and could lead to degradability being underestimated (Hungate, 1966; McDonald *et al*., 2002). Nel (2012) suggested that feed should be sieved through a 106 µm mesh before doing *in situ* trials, as it removes the small particles that would escape through the bag pores. It was also found that sieving had no effect on the chemical composition of soybean oilcake milled through a 2 mm screen. Further research (C.W. Cruywagen, University of Stellenbosch, 2007, unpublished data, as cited by Nel, 2012) found that the bags with an advertised mean pore size of 53 µm actually had pore sizes ranging from 31 to 99 µm, with a mean value of 63 µm, which could lead to an overestimation of 0 hour values. Nel (2012) further raised the concern that protein nutrition is very complex and that it is difficult to determine the different nitrogen fractions accurately, as results from the same study using different methods to obtain crude protein solubility showed great variation (Van de Haar & St-Pierre, 2006).

Although soybean oilcake meal was chosen as the model in this study, because of its popularity in animal nutrition, different results might have been observed if a different protein source was used. Soybean meal undergoes pre-treatment that involves heat application, making any additional heating less pronounced (Solanas *et al*., 2008). Soybean meal (which does not specify what previous processing it has undergone, could be full fat soya or oil expelled), however, should not be confused with soybean oilcake meal (which is what is left over after the oil has been extracted, pressed or solvent extracted), which differ in fat and protein content. Solanas *et al*. (2008) argued that heat treatment during oil extraction leads to reduced protein solubility, which could have been seen with the soybean oilcake meal used in this study, as the previous processing conditions it has undergone are not known. Cruywagen (Pers. Comm., November 2019) stated that soybean oilcake meal in general is known to give inconsistent results with *in situ* studies, as it sometimes does not reach an asymptote and thus the equation proposed by Ørskov & McDonald (1979) could not always be applied. It appears that degradation in this study was still increasing at the end of the incubation period at 48 hours. It might be beneficial in future studies to include a 72 hour incubation period to perhaps allow the model to reach an asymptote. Griffiths (2004) reported the same effect with soybean oilcake meal when the actual degradation value at 16 hours were compared and it was found that soybean oilcake meal extruded at 115-120 ºC reduced rumen degradation by 47.9%. Nel (2012) compared 12-hour incubation periods in dairy cows, which represents a passage rate of 8% using soybean oilcake that was either unsieved and sieved (106 µm). Both degradation values found were higher (65.3% and 56.6 %) than values obtained by this study, where the lowest disappearance value was 29.9% at 8 hours and the highest was 46.5% at 16 hours. The aim of this study was to compare the rumen degradability of the feeds, and all treatments were handled the same throughout the study. The *in situ* method is still a suitable method for the purpose of this study even though no significant differences were found.

In previous studies it was shown that heat treatments, including autoclaving and extrusion at temperatures from 100 ºC to 150 ºC, showed a decrease in rumen degradability of soybean meal and soybean oilcake meal, thus increased RUP fraction (Ljøkjel *et al*., 2000; Griffiths, 2004; Solanas, 2008). It is well known that chitosan is sensitive to temperature changes (Szymańska & Winnicka, 2015) and because soybean oilcake meal has already undergone processing, it is more prone to overheating and heat damage during extrusion, thus cold extrusion was used in this study. Literature on extrusion of feed below 100 ºC on the RUP fraction of plant protein sources is scarce. Thus, it was expected that no significant difference would be found with the use of cold extrusion. Some studies, however, found no effect of heat treatment or extrusion even at high temperatures (above 100 ºC) of soybean meal and soybean oilcake meal (Keery *et al*., 1993; Deacon *et al*., 1988). Prestløkken (1999) found that expansion at 150 ºC (5000 kPa) reduced the rumen degradation of soybean meal protein after 2, 4, 8, 16 and 24 hours of incubation but no differences was seen at 0 and 48 hours. Solanas *et al*. (2008) did not find any differences in rumen degradability during the first 4 hours of incubation and only found lowered crude protein degradability from 8 to 24 hours of

incubation. It is difficult to keep the conditions constant during the extrusion process and therefore it is not uncommon to get inconclusive results from extrusion studies and rumen degradability.

The mode of action of chitosan in the rumen is not completely understood. Del Valle *et al*. (2017) found that the addition of chitosan increased feed conversion efficiency and it was speculated that chitosan could possibly increase ruminant intestinal membranes permeability and thus increasing nutrients digestibility. The antimicrobial activity of chitosan could decrease microbial protein in ruminants (Gandra *et al*., 2016). Thus, when protein from feed is more digestible than those from microbial origin, amino acid uptake by the small intestine may be increased (McGuffey *et al*., 2001; Ruiz *et al*., 2001). Very few *in situ* studies have been done to determine the effect of chitosan on the rumen degradability of certain feeds, in order to compare it to the results found in this study. Of the previous studies done with chitosan, only a few found no differences for crude protein digestibility in sheep and heifers respectively (Goiri *et al*., 2010; Henry *et al*., 2015). Goiri *et al*. (2009b, c) found decreased ruminal protein disappearance and apparent total tract crude protein disappearance with addition of chitosan. However, Araújo *et al*. (2015), Mingoti *et al*. (2016) and De Paiva *et al*. (2017) reported an increase in apparent total tract crude protein digestibility in steers and cows with chitosan dosed through the cannula. The dose of chitosan tested in cows to probably give positive results with regards to rumen fermentation has been found to be between 0.01% and 0.02% of bodyweight (Mingoti *et al*., 2016; De Paiva *et al*., 2017). However, Del Valle *et al*. (2017) and Dias *et al*. (2017) found increased crude protein digestibility with the addition of chitosan at 0.4% (dry matter) to diets of Holstein cows and 0.12% (dry matter) for steers, respectively. The effect of chitosan on the rumen microbial population depends on the source, purity, dose, process of extraction and storage of the product (Jiménez-Ocampo *et al*., 2019). Thus, the anti-microbial activity of different chitosan types needs to be assessed prior to its utilisation as a feed additive (Belanche Garcia *et al*., 2016). Chitosan extracted from various sources differ significantly in the terms of molecular weight, degree of acetylation, purity level and moisture content, which is internal factors (Szymańska & Winnicka, 2015). Additionally, there also exist external factors that could lead to chitosan having different characteristics, such as its high susceptibility to environmental factors and processing conditions, including humidity, temperature and especially heating, which impose stress on its structure and can cause polymer degradation (Szymańska & Winnicka, 2015). Strong intermolecular interactions between formed fragments of chitosan (interchain crosslinking) alter the polymer structure, thus leading to the irreversible loss of its physiochemical properties (Szymańska & Winnicka, 2015). Chitosan with a higher molecular weight is more stable, especially thermal stability, and a higher degree of deacetylation has a less porous structure and a lower water-uptake ability, which limits the rate of the degradation process in an acidic environment (Szymańska & Winnicka, 2015). Therefore, chitosan with a higher degree of deacetylation favours the interaction between chitosan glucosamine radicals and components of the bacterial cell wall (Chung *et al*., 2004), which could reduce ruminal digestion.

Storage of chitosan solution at an elevated ambient temperature of 40 ºC resulted in faster degradation of chitosan chains (Nguyen *et al*., 2008). Therefore, it could be possible that extrusion of chitosan in this study, even at a low temperature of 45 ºC, could have defeated the purpose as it might have diminished the positive characteristics because it is sensitive to heat and pressure. The timing of gentle heating necessary to dissolve chitosan in an acidic solution should be carefully controlled, as overheating chitosan might cause polymer discolouration as a result of the depolymerization process. The rate of polymer damage also accelerates with rising temperature and duration. (Toffey *et al*., 1996). The addition of molasses with chitosan could have decreased the desired effect, as Szymańska & Winnicka (2015) reported that the inclusion of reducing sugars with chitosan could increase the Maillard reaction and form coloured products. In this study, there does not seem to be any benefits deriving from the addition of molasses. The heat of the extrusion was perhaps too low to have any effects.

There is a need to identify chitosan with specific characteristics that can give consistent results especially for ruminant animals, meaning that the chitosan should have stable predictable characteristics. A further problem is that specification data of chitosan from suppliers are often incomplete, which may be misleading. The intended use of chitosan as feed additive should specify if their purpose is to inhibit fermentation completely by decreasing feed digestibility or to decrease only the rumen degradability thereof, as this would affect the characteristics of the chitosan and potentially the dose needed to obtain the desired result (Goiri *et al*., 2009b). The rate of passage or retention time in the rumen has an influence on the extent of particle degradation in the rumen. Dufreneix *et al*. (2019) suggested that a density of 1.2 to 1.3 seems optimal to minimize retention in the rumen and large particles degrade more slowly. Thus, a particle with a density of 1.2 to 1.3 and diameter of 3 to 4 mm has a higher probability of escaping the rumen less degraded. Therefore, protein sources could perhaps be sieved and only the larger particles could be coated with chitosan to increase the chance of increasing the RUP thereof. Future studies could look at dissolving chitosan in a different acid, as Akhonkhai *et al*. (2006) found that chitosan-alginate microcapsules had better protein retention when dissolved with tartaric acid compared to the generally used acetic acid. Future studies could also look at encapsulating proteins or amino acids in chitosan, as Chiang *et al*. (2009) has coated amino acids with a simple coating of chitosan which was stable in the simulated rumen conditions at room and body temperature, while it released the amino acids slowly after time.

It is suspected that the soybean oilcake meal with molasses itself and / or the combination thereof with cold extrusion could have diminished the results expected from chitosan addition. More research is encouraged to investigate the addition of chitosan to protein sources for ruminant feeds. This study showed no differences in the RUP fraction of soybean oilcake, with molasses, processed by cold extrusion nor by the addition of chitosan. More research is still required in order to fully

understand the mode of action of chitosan within the rumen, the dose needed to elicit a specific response and the bioavailability of the undegraded protein in the small intestine.

5.6 Conclusion

This study revealed no improvement in the RUP fraction of soybean oilcake meal with 6% molasses when processed by cold extrusion or when 1% chitosan was added. The benefits of extrusion could not be reached with soybean oilcake meal and cold extrusion as applied in this study. More research is needed to fully understand the mode of action of chitosan in the rumen, the dose needed to elicit a response and the bioavailability of the protein thereof in the small intestine. Future studies should test the addition of chitosan on a different protein source and perhaps in a dose response manner, as the optimal dose has not been established. Different methods of incorporating chitosan to feed should be explored, such as spraying on pellets, mixed into feed as granules or possibly being mixed into licks. Literature shows that chitosan has much potential and should be further explored in ruminant nutrition.

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Chapter 6
The effect of extrusion with molasses and addition of tannins on the rumen undegradable protein fraction of soybean oilcake meal

6.1 Abstract

The process of extrusion and the addition of tannins have shown the potential to reduce the rumen degradability of soybean oilcake meal, thereby increasing the nutritional value thereof to ruminants. The aim of this study was to evaluate whether cold extrusion and the addition of hydrolysable tannins and the combination thereof, would affect the rumen undegradable protein fraction of soybean oilcake meal with molasses. Soybean oilcake meal was mixed with 6% molasses before the addition of tannins and extrusion. One percent sweet chestnut tannin extract was thoroughly hand mixed with the soybean oilcake meal. Cold extrusion was performed at 40-45 °C with a custom made cold, single screw extruder. Four Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula, were used in this trial. Samples were incubated in the rumen of the sheep in polyester bags at intervals of 0, 2, 4, 8, 16, 24 and 48 hours. The *in situ* study was carried out as a cross-over design with four treatments and four sheep over four periods. The actual rumen disappearance values were compared at each incubation timepoint. This research showed no differences with cold extrusion or the addition of 1% hydrolysable tannins on the rumen undegradable protein fraction of soybean oilcake meal with molasses. The benefits of extrusion could not be reached at low temperatures. The use of tannins has previously shown great potential as a feed additive by binding protein. However, more research is needed to fully understand the mode of action of tannins in the rumen and the bioavailability of bound protein in the small intestine.

6.2 Introduction

Nutritionists are continuously researching products that could optimise animal production traits by improving the diets of the animals and enhancing the value of feed ingredients, while reducing environmental impact and keeping costs as low as possible (Haraki *et al*., 2018). Protein is one of the most expensive nutrients. Therefore, it is essential to pursue the efficiency of protein utilisation. One of the main problems with high producing ruminants is the excess of rumen degradable protein (RDP) and a deficiency of rumen undegradable protein (RUP) content and so achieving the ideal ratio of RDP:RUP (Davidović *et al*., 2019). One way of improving nitrogen and thus protein efficiency may be to reduce dietary protein degradation in the rumen, thereby increasing the proportion of RUP, also called bypass protein. Therefore, by protecting the protein from degradation in the rumen, would increase the supply of amino acids to the small intestine and could reduce nitrogen wastage through

excretion in urine (Mohamaden *et al*., 2020). Soybean oilcake meal is the most common plant protein source used in livestock diets because of its high protein content (52.6% DM, INRA-CIRAD-AFZ Feed Tables, 2020) and favourable amino acid composition (Tona, 2018). The potential exists to further process soybean oilcake meal to reduce its rumen degradability for an improved quality protein source to ruminants. Chemical and heat treatments are the most common methods used for protecting dietary proteins. However, currently there is renewed interest in natural additives to modify rumen digestion due to increasing consumer demand for natural food products (Patra & Saxena, 2009).

Tannins are complex, naturally occurring plant polyphenolic compounds that have the potential to protect proteins from ruminal degradation and to decrease the rate of ammonia build-up in the rumen, making them a suitable additive in ruminant diets (Henke *et al*., 2017; Aderao *et al*., 2020). Tannins can form reversible bonds with proteins that are stable within the rumen pH range (5.5 to 7.0), making them less susceptible to degradation in the rumen by inhibiting the growth and activity of proteolytic bacteria, thus increasing the quantity of proteins that reach the abomasum and small intestine (Patra & Saxena, 2011; Henke *et al*., 2017; Patra & Aschenbach, 2018; Davidović *et al*., 2019; Sarnataro & Spanghero, 2020). The tannin-protein bond is believed to segregate at low pH, which occur in the acidic abomasum or in the duodenum and therefore allow a higher absorption of proteins and amino acids in the intestine (Chalupa, 1975; Jones & Mangan, 1977). The suppressing effect of tannins on the rumen microbiome links its value to environmental issues, not only through reducing nitrogen pollution, but also decreasing methane emissions from rumen fermentation (Patra & Saxena, 2011; Patra & Aschenbach, 2018; Sarnataro & Spanghero, 2020). The effect of tannins on the rumen microbiome and the protein binding capacity depends on the structure and source of tannins, as well as the plant protein source (Giner-Chavez *et al*., 1997; Kraus *et al*., 2003; Zeller *et al*., 2015). Tannins could be classified into two main groups, namely condensed tannins and hydrolysable tannins, which are different in structure and molecular weight depending on the origin (Mohamaden *et al*., 2020; Sarnataro & Spanghero, 2020). Condensed tannins are the most intensively studied for its use in decreasing rumen degradable protein fractions and improving nitrogen utilisation, but also for reducing bloat and parasitism in ruminants and reducing methane emissions (Coblentz & Grabber, 2013). The concern with condensed tannins is that the bond with proteins might sometimes be irreversible as it is more stable in the rumen environment and not degraded by natural processes, rendering the protein unavailable for absorption in the small intestine (Archana *et al*., 2010; Mezzomo *et al*., 2015). Hydrolysable tannins have a weaker bond with proteins and it may be degraded with metabolites being absorbed into the bloodstream, which could lead to toxicity (Khanbabaee & Van Ree, 2002; Aboagye & Beauchemin, 2019). However, there are studies showing no detrimental effect of using hydrolysable tannins in ruminant diets and some authors recorded that there were no differences in rumen protein degradation between different tannin sources (Driedger & Hatfield, 1972; Getachew *et al*., 2008; Liu *et al*., 2011). It has been shown that high tannin content (>5% DM) reduces voluntary intake and nutrient digestibility to a great extent

through decreased feed palatability and slower digestion (Frutos *et al*., 2004a; Mueller-Harvey, 2006). By contrast, the intake of low to medium tannin content (1-4% DM) has been shown to improve feed conversion and digestion mainly due to decreased ruminal protein degradation (Mueller-Harvey, 2006; Patra & Saxena, 2011). However, the effect of tannins on protein degradability is inconsistent.

Several authors found positive effects such as increased milk yield and milk protein, as well as decreased milk urea nitrogen concentration, in dairy cows using condensed tannins (Dey & De, 2014; Wang *et al*., 1996; Soltan, 2009; Allam *et al*., 2013; Anantasook *et al*., 2015). Others found that the inclusion of 2-4% DM condensed tannins (Piñeiro-Vázquez *et al*., 2017) or hydrolysable tannins (Wischer *et al*., 2014) did not affect protein utilisation efficiency in cattle and sheep, respectively. Arisya *et al*. (2019) found that tannins from various sources decreased dry matter and rumen degradable protein, but it did not affect dry matter digestibility or crude protein digestibility and concluded that 2% chestnut tannin gave the best results. Availability of literature on condensed tannins is extensive, whereas hydrolysable tannins have been less studied in ruminant nutrition.

Extrusion is a process in feed manufacturing where heat and pressure are applied in the presence of moisture, most commonly for oil extraction, but also more recently to decrease rumen degradability of protein sources (White *et al*., 2007; Zagorakis *et al*., 2015). The raw material is fed through a barrel with increasing pressure as the barrel tapers toward the outlet. This method of protecting proteins is considered safe and economical. Extrusion causes denaturing of proteins, which decreases protein solubility and thus also decreases the ruminal degradability of protein in feeds (Barchiesi-Ferrari & Anrique, 2011). The RUP fraction is thus increased, providing greater quantities of amino acids available for absorption (Solanas *et al.,* 2008). Extrusion at 116 ºC with the addition of molasses has been shown in Chapter 3 and 4 to decrease rumen degradability of lupins and canola oilcake meal. The effect of extrusion could be enhanced by addition of molasses due to the partial Maillard reaction during the addition of heat (Chalupa, 1975; Solanas *et al*., 2008). Cold extrusion was chosen for this study to prevent potential heat damage to already extruded soybean oilcake meal. Furthermore, information on the effect of cold extrusion on the rumen degradability of protein sources is scarce in literature. Different treatments of extrusion have been described, but studies combining the effects of tannins and extrusion with molasses is rare.

Therefore, the aim of this study was to determine the effect of cold extrusion and the addition of hydrolysable, sweet chestnut tannins on the *in situ* rumen degradability of soybean oilcake meal with molasses.

6.3 Materials and Methods

Animals and diets

Ethical clearance for this research was granted by the Research Ethics Committee for Animal Care and Use of the Stellenbosch University (Ethical clearance numbers #0378 and #0379). Four Dohne Merino wethers weighing ±80 kg, fitted with rumen cannula, were housed in enclosed individual pens (1 m x 2 m) at the Welgevallen Experimental Farm of the Stellenbosch University. The sheep had *ad libitum* access to clean drinking water and were supplied a basal diet of wheat straw and lucerne hay (50:50) *ad libitum* during the experimental period. The feed troughs were filled twice daily (every morning and evening) as necessary. Daily intake was estimated as 3% of the body weight of the sheep. The sheep were already adapted to the feed before the *in situ* trial started.

Treatments

Soybean oilcake meal was thoroughly mixed with 6% molasses powder (Kalori 3000, Yara Animal Nutrition). One portion of the soybean oilcake meal with molasses was kept as a control and another portion was thoroughly hand mixed with 1% sweet chestnut tannin extract (55% active substance, sucrose, water and 2% water-insoluble impurities). Both soybean oilcake meal treatments were dried in a force draught oven overnight at 50 °C. Half of the soybean oilcake meal with molasses, and half of the soybean oilcake meal with molasses and tannins was milled through a 2 mm Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) before being cold-extruded at 40-45 °C with a custom made cold, single screw extruder (Nutritionhub Pty Ltd, Stellenbosch, South Africa). After extrusion, the treatments were dried at 60 ºC for 4 hours to reach a 3% moisture content.

The four feeds tested in the trial were soybean oilcake meal with molasses not extruded, without tannins (SOM), soybean oilcake meal with molasses extruded, without tannins (SOME), soybean oilcake meal with molasses and tannins, not extruded (SOMT), and soybean oilcake meal with molasses and tannins, as well as extruded (SOMTE). All four feeds were milled through a 2 mm screen size using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) for the *in situ* analyses, according to NRC recommendations (NRC, 2001).

In situ evaluation of ruminal degradability

The dry matter (DM) and crude protein (CP) degradabilities of SOM, SOME, SOMT and SOMTE were determined by the *in situ* technique described by Ørskov & McDonald in 1979. The feed samples were dried in a force draught oven for a minimum of 48h at 60 °C, after which 5 g samples were weighed off on a digital scale and inserted into each of a series of previously dried, weighed and marked polyester bags (12 cm x 9 cm) with mean pore size of 15 µm. The bags were tied off by nylon strings of different lengths to prevent the strings from knotting in the rumen, as well as to ensure easy retrieval of the bags. Bags were incubated in the rumen at different time intervals, being 2, 4, 8, 16, 24 and 48 hours with an all-out approach. An incubation series started when the first bag was inserted into the rumen cannula at 14h00 in the afternoon. The consecutive bags were

inserted at following times to achieve the respective incubation periods. The incubation period was ended when all the bags were removed at the same time point after 48h. After bag removal, the bags were submerged in ice water to rapidly stop further microbial degradation. The bags were washed under running tap water until water squeezed from it was clear. The 0h bag was prepared in the same way and was washed under the tap until clear like the rest, without being placed in the rumen. All bags were dried after incubation in a force draught oven for a minimum of 48h at 60 °C.

This procedure was carried out in a cross-over design where each of the four feeds was tested in each of the four sheep over four periods (one feed per sheep per period), giving a total of four observations for each variable studied.

Chemical analysis

After drying the bags for 48 hours at 60 °C, the nylon strings were removed, and the dried bags were weighed to determine the DM residue. The nitrogen content (%) of the residue was then determined using the Dumas combustion method (Method 990.03; AOAC, 2002) using a LECO TruMac N Nitrogen Determinator, version 1.3X (LECO Corporation, Michigan, USA). The CP content of the dry matter was determined by multiplying the percentage nitrogen by a factor of 6.25.

The chemical properties of SOM, SOME, SOMT and SOMTE before rumen incubation were determined with the official methods as described by the Association of Official Analytical Chemists (AOAC, 2002) for DM (method 934.01), ash (method 942.05), CP (method 990.03), and crude fat (method 2003.06). NDF and ADF were determined according to Van Soest *et al*. (1991), and calcium and phosphorus by method 6.1.1 of the Agri Laboratory Association of Southern Africa guidelines (ALASA, 1998).

Statistical analysis

Dry matter and CP disappearances were expressed as percentages of the amount of treatment remaining in the bag after rumen incubation. The percentage material degraded did not fit the non-linear regression one-compartment model, as proposed by Ørskov & McDonald (1979). This might have been due to particle sizes varying between treatments or samples, as extrusion and milling of already extruded oilcake meals could have led to very fine particle sizes and thus an asymptote has not been reached within the 48 hour incubation period. The percentage particle distribution was determined by the fineness modulus (ASTM C136 / C136M – 19, 2019) and can be seen in Table 6.1. Five gram samples of each of the treatments were manually put through the stack of sieves (Labotec sieves with sizes 1.18 mm, 850 µm, 250 µm, 180 µm, 150 µm, 125 µm, 106 µm and <106 µm) three times and the average was used to calculate the fineness modulus.

For this reason, the actual disappearance values obtained at each timepoint was submitted to an ANOCOVA with the 0h value as the covariate in order to adjust for the starting value of each treatment as interaction was found at 0h (representing the readily soluble particles and particles that was small enough to escape through the pores of the bag), using SAS 9.4 software (SAS Institute

Inc., Cary, NC, USA, 2014). Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$ using Bonferroni tests.

6.4 Results

The fineness modulus and particle distribution of SOM, SOME, SOMT and SOMTE are presented in Table 6.1. The fineness modulus for SOM and SOME were the same at 6.0 while the samples with tannins have a slightly lower fineness modulus at 5.8 and 5.7 for SOMT and SOMTE, respectively. Extrusion led to a bigger proportion of finer particles as the proportion left on the pan (<106 µm) was 5.0% and 7.9% for SOME and SOMTE compared to 2.5% and 3.6% for SOM and SOMT, respectively. Extrusion also slightly increased the proportion large particles left on the 1.18 mm sieve, which were 2.6% and 1.7% for SOME and SOMTE, respectively, compared to 0.4% for both SOM and SOMT. The same trend could be seen on the 850 µm sieve. It, therefore, seems as if the extrusion process led to an increased proportion of bigger as well as finer particles.

Table 6.1 The fineness modulus of soybean oilcake meal (SOM), soybean oilcake meal extruded (SOME), soybean oilcake meal with tannins (SOMT) and soybean oilcake meal with tannins and extruded (SOMTE) (all treatments with addition of 6% molasses)

	Cumulative percentage retained on each sieve			
Sieve size	SOM	SOME	SOMT	SOMTE
1.18mm	0.4	2.6	0.4	1.7
850µm	14.9	31.6	15.4	20.9
250µm	93.5	90.0	88.8	83.6
180µm	95.9	92.8	93.1	88.1
150µm	96.7	93.6	94.6	89.8
125µm	97.0	94.1	95.3	90.8
106µm	97.5	95.0	96.4	92.1
<106µm	100.0	100.0	100.0	100.0
Fineness modulus	6.0	6.0	5.8	5.7

The chemical composition of the treatments used in this study is presented in Table 6.2. It seems that the extrusion process led to a higher dry matter content. The extruded samples seem to have a slightly higher ash, crude protein and NDF content compared to not extruded, although the significance was not tested.

Table 6.2 The chemical composition of soybean oilcake meal with an addition of 6% molasses either with or without 1% tannins added and either extruded or unprocessed. All values (except DM) are expressed on a DM basis

Feed	DM (%)	Ash (%)	CP (%)	CF (%)	NDF (%)	ADF (%)	Ca (%)	P (%)
Soybean oilcake meal	86.3	7.9	43.8	0.8	6.9	4.6	0.7	0.7
Soybean oilcake meal extruded	95.9	8.5	47.6	0.6	9.0	5.7	0.8	0.8
Soybean oilcake meal with tannins	87.5	7.9	42.7	0.7	6.5	4.6	0.9	0.7
Soybean oilcake meal with tannins extruded	95.7	8.7	46.2	0.7	9.4	5.0	0.9	0.8

DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fibre, ADF = acid detergent fibre, Ca = calcium, P = phosphorus

The actual dry matter disappearance values at the different incubation times in the rumen are presented in Table 6.3. No interaction was observed between processing (extrusion) and polyphenol addition (tannins). Thus, the main effects could be interpreted separately. No differences were observed between the main effects for dry matter disappearance from the rumen.

Table 6.3 The effect of extrusion and addition of tannins on the actual *in situ* dry matter disappearance values (%) at 2, 4, 8, 16, 24 and 48 hours rumen incubation time of soybean oilcake meal with molasses

		*Actual dry matter disappearance values at incubation time (%)					
		2h	4h	8h	16h	24h	48h
Processing	Without extrusion	37.8 ± 0.8	40.6 ± 1.7	49.1 ± 5.0	62.0 ± 7.6	87.5 ± 6.4	99.0 ± 3.3
	With extrusion	39.4 ± 0.8	44.1 ± 1.7	56.3 ± 4.5	72.8 ± 7.6	79.5 ± 6.4	93.1 ± 3.3
	P-value	0.625	0.568	0.330	0.382	0.507	0.451
Polyphenol	Without tannins	38.7 ± 0.4	42.0 ± 0.8	54.2 ± 2.1	69.5 ± 3.4	84.9 ± 2.9	95.2 ± 1.5
	With tannins	38.4 ± 0.4	42.7 ± 0.8	51.2 ± 2.2	65.2 ± 3.4	82.2 ± 2.9	96.8 ± 1.5
	P-value	0.313	0.295	0.446	0.466	0.523	0.358

*Least square mean ± standard error, n = 8
The 0 hour disappearance value was used as a covariate

The actual crude protein disappearance values at the different incubation times in the rumen are presented in Table 6.4. Again, no interaction was observed between processing (extrusion) and polyphenol addition (tannins). Thus, the main effects could be interpreted separately. No differences were observed between the main effects for crude protein disappearance from the rumen.

Table 6.4 The effect of extrusion and/or addition of tannins on the actual *in situ* crude protein disappearance values (%) at 2, 4, 8, 16, 24 and 48 hours rumen incubation time of soybean oilcake meal with molasses

		*Actual crude protein disappearance values at incubation time (%)					
		2h	**4h**	**8h**	**16h**	**24h**	**48h**
Processing	Without extrusion	20.7 ± 1.0	23.8 ± 1.1	32.9 ± 3.2	48.8 ± 5.2	73.6 ± 5.7	94.0 ± 2.9
	With extrusion	22.7 ± 1.0	27.1 ± 1.1	32.4 ± 2.9	49.1 ± 5.2	74.1 ± 5.7	95.0 ± 2.9
	P-value	0.228	0.761	0.425	0.905	0.508	0.312
Polyphenol	Without tannins	22.6 ± 0.9	25.7 ± 1.0	34.3 ± 2.6	49.4 ± 4.7	76.4 ± 5.1	92.5 ± 2.6
	With tannins	20.9 ± 0.9	25.2 ± 1.0	31.0 ± 2.9	48.5 ± 4.7	71.3 ± 5.1	96.5 ± 2.6
	P-value	0.240	0.086	0.926	0.966	0.468	0.845

*Least square mean ± standard error, n = 8
The 0 hour disappearance value was used as a covariate

6.5 Discussion

With regards to the chemical composition of the treatments in this study, a higher dry matter content for extruded feeds was expected, as the feeds were dried to a moisture content of 3% as part of the extrusion process. No differences in dry matter or crude protein disappearances were observed for cold extrusion nor the addition of 1% hydrolysable sweet chestnut tannin extract treatments. Different aspects were incorporated into this study, including the *in situ* method used, the use of already processed soybean oilcake meal, cold extrusion, the use of hydrolysed sweet chestnut tannins, and the addition of molasses, which could have had an influence on the results obtained.

The *in situ* method is a commonly accepted method to evaluate rumen degradability, but it is not without shortcomings. The method is known to overestimate the degradability of feeds, because of small particles that escape through the bag pores. Some of the factors that have the greatest influence on the *in situ* method were kept constant throughout the trial, including bag characteristics (pore and bag size), the animals used (species and diet) and to a lesser extent the environment (Michalet-Doreau & Cerneau, 1991). Another characteristic that could have had a major influence on the *in situ* method results, which could not be controlled beyond methodology, is the particle size of samples from the different treatments. The particle distribution within the fineness modulus (Table 6.1) shows that the extrusion process as applied in this study could have led to an increased proportion of larger and finer particles, which is in agreement with results obtained in Chapter 5. It can be expected that there will be considerable differences in particle size between the same feeds, ground with the same screen. Therefore, it has been suggested that degradability studies should rather be based on particle size rather than the screen size of milling (Michalet-Doreau & Cerneau, 1991). Nel (2012) suggested that feed should be sieved through a 106 µm mesh before doing *in situ* trials, as it removes the small particles that would escape through the bag pores. It was also found that sieving had no effect on the chemical composition of soybean oilcake milled through a 2 mm screen. After further research it was found that the bags with an advertised mean pore size of 53 µm had pore sizes ranging from 31 to 99 µm, with a mean pore size of 63 µm, which could lead to an

overestimation of degradation at 0 hour (C.W. Cruywagen, Stellenbosch University, 2007, unpublished data, as cited by Nel, 2012). Nevertheless, it is evident that the particle distribution differed between feeds tested in this study and it justifies the use of the 0 hour value as covariate, as this corrects for the differences in fine particles and potentially the solubility thereof. The grinding of the feed used in the different treatments before being used in *in situ* studies is inevitable and is necessary to reduce the degree of variation and to simulate mastication of feed, which is normal before digestion (Michalet-Doreau & Cerneau, 1991).

Although soybean oilcake meal was chosen as the model in this study because of its popularity in animal nutrition, different results might have been observed if a different unprocessed protein source was used. Cruywagen (Pers. Comm. November, 2019) stated that soybean, in general, is known to give variable results with *in situ* studies as it sometimes does not reach an asymptote and thus the equation proposed by Ørskov & McDonald (1979) could not always be applied. As it appears that degradation was still increasing at the end of the incubation period at 48 hours in this study, it might have been beneficial to include a 72 hour incubation period to perhaps allow the model to reach an asymptote. The aim of this study was to compare the rumen degradability of the different treatments, and all treatments were handled the same throughout the study. Therefore the *in situ* method is a suitable method for the purpose of this study even though no significant differences between treatments were found.

In previous studies it was shown that heat treatments, including autoclaving and extrusion at temperatures from 100 ºC to 150 ºC, showed a decrease in rumen degradability of soybean meal and soybean oilcake meal, thus increasing the RUP fraction thereof (Ljøkjel *et al*., 2000; Griffiths, 2004; Solanas, 2008). The extrusion conditions in this study were most likely at a too low temperature to cause differences in rumen degradability. There are very few studies that have combined extrusion and the addition of tannins. Dhumez *et al*. (2018) used a faba bean and rapeseed blend with 0.2% inclusion of hydrolysable chestnut tannins and found a slightly decreased crude protein effective degradability with extrusion at 140 ºC, while not observing a difference during extrusion at 45 ºC or 60 ºC. This could also support the view that the temperatures during cold extrusion were too low to elicit a positive response. Soybean oilcake meal has already undergone processing and thus it is more prone to overheating and heat damage during extrusion, therefore, cold extrusion was used in this study. Literature on the effect of extrusion of feed below 100 ºC on the RUP fraction of plant protein sources is scarce, as cold extrusion is mostly used in aquatic feeds and pet food. Some studies, however, found no effect of heat treatment or extrusion on degradability, even at high temperatures (above 100 ºC) of soybean meal and soybean oilcake meal (Keery *et al*., 1993; Deacon *et al*., 1988). It is difficult to keep the conditions constant during the extrusion process and therefore, it is not uncommon to get inconclusive results from rumen degradability of extruded feed.

Hydrolysable tannins are still widely viewed as being toxic to ruminants, even at low doses. This could be one possible reason for the literature being scarce on the use of hydrolysable tannins

as a feed additive for reducing protein degradation in the rumen. However, it has been shown that inclusion levels up to 4% are safe and studies with hydrolysable tannins have been shown to decrease ruminal degradation of soybean meal without detrimentally affecting its intestinal digestion and absorption in sheep (Hervás *et al*., 2000). Several authors found reduced protein degradation and positive effects in the production of ewes and dairy cows, when using tannins (Wang *et al*., 1996; Min *et al*., 2003; Frutos *et al*., 2004a, b; Soltan, 2009; Allam *et al*., 2013; Dey & De, 2014; Anantasook *et al*., 2015; Davidović *et al*., 2019). However, some authors did not find significant effects with the use of tannins in feed for cows, especially on the feed intake, milk yield, protein content and composition, lactose, fat content and fatty acid profile in milk (Benchaar & Chouinard, 2009; Aguerre *et al*., 2016; Broderick *et al*., 2017).

Even though no rumen degradability differences due to extrusion or the addition of hydrolysable tannins were found in this study, a possibility exists that there could have been other effects that were not measured. Mohamaden *et al*. (2020) added on average 0.8% hydrolysable tannins to alfalfa in the feed of rams and did not find any differences in the *in situ* RUP of soybean meal. Higher blood serum total protein and an increased average weight gain at 30 days from the start of the experiment compared to 10 and 20 days were, however, found. A possible reason for the difference in results between days might be that the animals could need an adjustment period to tannins before positive effects can be seen. Similarly, Aderao *et al*. (2020) supplied feed with 0.9% condensed tannins to growing lambs and found no differences in dry matter intake, live weight gain and nutrient digestibility, but less nitrogen was excreted through the urine, indicating better utilisation of absorbed nitrogen. Jolazadeh *et al*. (2015) did not find differences in CP digestibility, but found decreased rumen ammonia concentration and protozoal population, along with increased average daily gain and better feed conversion efficiency with the addition of tannins to the feed of Holstein bulls. Other studies indicated that even at higher inclusion levels of 2-4% DM, the protein utilisation efficiency of cattle and sheep was not affected by either the addition of condensed tannins (Piñeiro-Vázquez *et al*., 2017) or hydrolysable tannins (Wischer *et al*., 2014) to their feed. The inclusion level of tannins in this study was perhaps too low to elicit the desired response, as the active substance is 0.55% of the 1% inclusion of sweet chestnut tannin extract. Mergeduš (2020) pointed out that in some studies, the tannin source and concentrations were not supplied accurately or with enough detail. Thus, the concentration of tannins could mostly have been too low to detect differences within studies. Lorenz *et al*. (2014) indicated that the type of protein source used could influence the concentration of tannins that is needed to cause the binding of proteins, proposing that a higher inclusion level of the tannin extracts may be required to decrease rumen protein degradation of the soybean oilcake meal. It is known that hydrolysable tannins are more susceptible to microbial hydrolysis in the rumen than condensed tannins (McSweeney *et al*., 2001; Ozdal *et al*., 2016). Therefore, rumen microbes may reduce or completely remove the effectiveness of hydrolysable tannins on protein degradability (Hervás *et al*., 2000; Salami *et al*., 2018).

Several *in vivo* studies support the results of the effect of low dose tannins in reducing protein degradation in the rumen (Puchala *et al*., 2005; Al-Dobaib, 2009; Broderick *et al*., 2017). Recently, Sarnataro & Spanghero, (2020) included 1.4% chestnut tannin in feed of dairy cows, which reduced *in vitro* protein degradation and rumen ammonia yield. Similarly, 2% inclusion of hydrolysable tannins by Frutos *et al*. (2004b) to concentrate feed for finishing lambs showed lower ruminal crude protein degradability, without a negative effect on performance. Similar results were found by Arisya *et al*. (2019) with 2% chestnut tannin inclusion, which decreased *in vitro* RDP (rumen fluid from crossbred cattle), but it did not affect total tract crude protein digestibility. This research indicated that the tannin was released post ruminally, leading to increased amino acid flow to the small intestine. Tabacco *et al*. (2006) showed that 4% chestnut tannin applied prior to alfalfa ensilage reduced proteolysis, but only a slight reduction in organic matter digestibility of feed in dairy cows was observed. Similarly, an *in vitro* study with 5% inclusion of chestnut tannin in feed reduced protein degradation, but it caused a slight negative impact on the total volatile fatty acid concentration (Hassanat & Benchaar, 2013). These results supported other studies indicating that a maximum of 4% tannins should be included in the feed. The response of rumen fermentation and feed digestibility is shown to vary between different tannin sources supplemented at similar levels (Makkar *et al*., 1995; Jayanegara *et al*., 2015). Thus, the optimal inclusion level or tannin source has not been identified yet. The optimal inclusion level of tannins can differ depending on the goal (reducing protein rumen degradation or reducing methane emission).

It has been suggested that the combination of condensed and hydrolysable tannins in feed is ideal for decreasing rumen protein degradation. Results by Aguerre *et al*. (2016) used a combination of quebracho and chestnut tannin extract (2:1) at 0.45% inclusion in the feed, which decreased urinary nitrogen excretion and CP digestibility in dairy cows. Mezzomo *et al*. (2015) found increased RUP of soybean meal with a 2.5% tannin mixture in the feed (85% condensed to 15% hydrolysable). Salami *et al*. (2018) included hydrolysable and condensed tannins at 4% in feed in a long-term feeding trial and found antimicrobial activity against methanogens and protozoa without compromising ruminal fermentation. Bueno *et al*. (2020) suggested that prolonged incubation duration of 96 hours is necessary to allow for adaptation of the rumen bacterial community to diets, while Waghorn (2008) emphasised the importance of microbial adaptation when using hydrolysable tannins. Thus, longer incubation periods could have been necessary to obtain significant differences in this study to allow for adaptation of the rumen microbiome.

Discrepancies in the results obtained may be explained by the different sources of tannins (structure and origin), dose and method of addition, animal species and physiological status, and diet composition (Makkar, 2003; Mueller-Harvey, 2006; Waghorn, 2008; Patra & Saxena, 2011; Davidović *et al*., 2019; Bueno *et al*., 2020). Bueno *et al*. (2020) found that condensed tannins had a greater effect on inoculum from cattle than from sheep. Furthermore, the comparison of the effect of tannins in feed across different *in vivo*, *in vitro* and *in situ* studies may be further complicated due to

differences in the basal diet of animals used and thus digestion kinetics (concentrate vs forage-based diet) and the ruminant species. Concentrate diets are rapidly digested in the rumen compared to forage-based diets and differences in rumen kinetics exist among ruminant species (Colucci *et al*., 1984; Huhtanen *et al*., 2006). These variations could temporarily limit the formation of tannin-protein bonds, especially in concentrate diets (Salami *et al*., 2018). Results obtained in different studies indicated that chestnut tannins did not affect ruminal proteolysis when supplemented in a concentrate diet of lambs and sheep (Salami *et al*., 2018; Wischer *et al*., 2014), but it reduced ruminal protein degradation when included in the forage-based diet of cattle (Tabacco *et al*., 2006).

In this study, there does not seem to be any benefits from the addition of molasses, as the heat of the extrusion was perhaps too low to have caused the desired Maillard reaction. The source of tannins used in this study already contains sucrose and the addition of molasses to feed used in future studies could be unnecessary, unless added to concentrate diets for lambs to increase palatability (Frutos *et al*., 2004b). Earlier studies focused on condensed tannins in forages (mostly legumes) and tree leaves, where interest is recently shifting more towards tannin extracts, especially hydrolysable tannins such as chestnut. Although there is substantial proof of benefits in literature with the addition of tannins on protein digestion, ruminant productivity and quality, there is a need to investigate their effectiveness in larger feeding trials, perhaps over longer periods and within different animal species. The source and dose of tannins to achieve positive results should be thoroughly tested. The variable results in literature with regards to tannin source and content indicate the necessity of further investigations to better understand the mode of action and potency of different tannins. Therefore, the future challenge will be to identify cost effective use of tannins and optimal doses, or perhaps the ideal combination of hydrolysable and condensed tannins for reducing rumen degradability of plant protein sources. Further investigation is required in the interaction between rumen microbes and hydrolysable tannins and the consequent effect on rumen fermentation. It is suggested that in future studies, different data measurements relating to nitrogen efficiency should be included alongside rumen degradability, including animal performance measurements, total tract digestibility, amino acid bioavailability, nitrogen excretion, methane production, as well as the influence on the rumen microbiome.

6.6 Conclusion

This study revealed no improvement in the RUP fraction of soybean oilcake meal (with 6% molasses) when processed by cold extrusion or when 1% hydrolysable sweet chestnut tannins were added. The benefits of extrusion could not be reached by processing conditions as applied in this study, neither by the addition of hydrolysable tannins to reduce rumen degradability. More research is needed to fully understand the mode of action of tannins in the rumen, together with knowledge of the rumen microbiome, tannin source, the dose needed to elicit a response and the bioavailability of the treated protein source in the small intestine. Even though no differences were seen in results

obtained in this study, literature shows that tannin has much potential and should be explored in ruminant nutrition. Future studies should test the addition of tannins to a different protein source and perhaps in a dose response manner, as the optimal dose has not been established. Rumen microbes exposed to tannins might also need an adaptation period to positively effect rumen degradation of proteins.

6.7 References

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Chapter 7
General conclusion and future prospects

The aim of this study was to increase the rumen undegradable protein (RUP) fraction of plant protein sources (lupins, canola oilcake meal and soybean oilcake meal). The applied methods were extrusion (hot and cold), addition of a polymer (chitosan) and polyphenols (hydrolysable tannins) to potentially increase the supply of amino acids to the small intestine for improved animal production.

Conclusions per objective

- Extrusion at 116 ºC was found to modify ruminal degradation parameters of broad-leaf *L. albus* and narrow-leaf *L. angustifolius*, while also decreasing the effective rumen degradation of the two lupin types. Thereby, the objective of increased RUP fraction of lupins was achieved with a reduction in crude protein rumen degradability of up to 28% at a high outflow rate. The effect of extrusion of the two lupin types on protein degradability seemed to be more pronounced at a faster outflow rate.
- Extrusion with molasses at 116 ºC was found to modify ruminal degradation parameters of canola oilcake meal and crushed sweet lupins, while also decreasing the effective rumen degradation, especially at faster outflow rates. Thereby, the objective of increased RUP fraction of canola oilcake meal and crushed sweet lupins was achieved by an 85.4% increase through extrusion with molasses compared to not extruded. This study showed that the benefits of extrusion could be reached by a relatively low temperature of 116 ºC with the addition of 6% molasses.
- For the third and fourth objectives no improvement was revealed in the RUP fraction of soybean oilcake meal with 6% molasses when processed by cold extrusion or when 1% chitosan or 1% hydrolysable tannins were added. The benefits of extrusion could not be reached with soybean oilcake meal and cold extrusion as applied in this study. The inclusion level of 1% chitosan or 1% hydrolysable tannins might have been too low to get the desired response.

Implications

Locally produced and properly processed plant protein sources, such as canola and lupins, play a vital role in supporting the livestock production sector, which is an important and reliable source of animal protein in human diets. This means that the use of canola oilcake meal and lupins, which previously have been limited in diets for high producing animals due to its high RDP content, could be included in diets at higher levels following the process of extrusion. Processing of canola oilcakes and lupins with extrusion increase the potential of these plant protein sources in supporting and enhancing animal production. Further, it decreases dependence on imports of raw materials and

feed ingredients for which the demand is increasing worldwide. The rumen degradability data obtained by the first and second trial may aid in more accurate formulation of ruminant diets using extruded lupins or canola oilcake meal. Emphasis should be put on processing conditions, method and precautions while the feed is made, otherwise, unsatisfactory results could be obtained. Soybean can give variable results, so care must be taken when using it.

Future prospects

Further research is required to examine the effect of extrusion with molasses of canola oilcake meal, crushed sweet lupins and soybean oilcake meal on the intestinal digestibility, particularly its effect on the availability of essential amino acids. This could be done through bioassays to be able to generate true ileal digestibility values of crude protein to be used in feeds. Further studies are needed on the extrusion conditions to determine optimum temperature and moisture during processing in order to optimise the RUP fraction. Further studies are also needed to determine the effect of feeding extruded lupins on the digestion and growth or production performance of ruminants.

Literature shows that chitosan and tannins have much potential and should be further explored in ruminant nutrition. More research is needed to fully understand the mode of action of chitosan and hydrolysable tannins in the rumen, the dose needed to elicit a response and the bioavailability of the protein thereof in the small intestine. Future studies should test the addition of chitosan and tannins on a different protein source and perhaps in a dose response manner, as the optimal dose has not been established. Different methods of incorporating chitosan and tannins to feed should be explored, such as spraying on pellets, mixed into feed as granules or possibly being mixed into licks. As it seems that tannins might need an adaptation period, the addition of tannin could be tested in digestibility trials with longer incubation periods and it is suggested to include different measurements regarding nitrogen utilisation in addition to rumen degradability. With regard to the addition of chitosan and tannins, perhaps the focus could be shifted from the different plant protein sources and rather to look at the effect on the rumen microbes.

The optimisation of the nutritional value of feed for livestock diets is of vital importance and should be advanced on an ongoing basis in order to contribute towards an optimised and sustainable food value chain. Enhanced knowledge and techniques in the agriculture industry will lead to improved economic farming in the interest of food security world-wide. Fertile land and resources are limited with increased environmental pressure; there is only one world to feed an increasing number of people.